Oscillatory Mechanisms of Memory Consolidation during Sleep

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II Summary

Sleep plays a critical role in memory consolidation. Memories are initially stored in the hippocampus and over time, through the process of consolidation, they become integrated into existing knowledge networks in the neo-cortex. Particularly the spontaneous reactivation of a memory trace during slow wave sleep (SWS) is thought to strengthen that specific trace and facilitate the transfer from a hippocampal to a cortex-dependent memory store. Different neuronal oscillatory events facilitate these processes of consolidation during sleep. This thesis presents four manuscripts related to the study of the oscillatory mechanisms underlying memory consolidation during sleep.

Manuscript I shows that targeted memory reactivation (TMR) during sleep leads to oscillatory changes in the human brain that persist during subsequent wake. Specifically, words that were reactivated during sleep show enhanced theta activity during recognition testing. This enhancement is located in the left inferior prefrontal cortex, an area associated with deeper word processing. Additionally, the study emphasizes the importance of theta activity in memory related processing in the brain.

Manuscript II discusses the importance of the interplay of slow oscillations (SOs) and sleep spindles for memory consolidation processes. Two auditory stimulus approaches that enhance the amount of slow wave activity are compared, one that produces enhanced memory consolidation and one that does not. Importantly, the approach that achieves increased memory performance also induces an increase in sleep spindles, while the other does not.

Manuscript III presents a closed-loop TMR approach to target auditory stimuli into the up- and down-states of a SOs. Cueing words into up-states enhances memory performance over uncued words. Down state cued words only show a marginal enhancement over uncued words. During sleep, the oscillatory response of up-state cued remembered words compared to non-remembered words shows an increase in both spindle power and theta after the cue. For down-state cued words, no such oscillatory difference can be found.

Manuscript IV presents an unsupervised TMR study over four days. Participants sleep at home and play an audio recording of word cues. Across the whole experiment period, there is no beneficial cueing effect on memory performance. Only on the third night, after subjective sleep quality has returned to normal levels, there is a beneficial effect of TMR.

In sum, this thesis provides evidence for the importance of SOs, sleep spindles and theta activity, and in particular their interplay, for memory consolidation processes. Additionally, the importance of the ‘when and how’ of memory consolidation is highlighted.

III Zusammenfassung

In der Gedächtniskonsolidierung spielt der Schlaf eine kritische Rolle. Durch den Prozess der Konsolidierung werden Erinnerungen, die zunächst im Hippocampus abgespeichert werden über die Zeit in bereits existierende neokortikale Wissensnetzwerke überführt. Es wird angenommen, dass insbesondere die spontane Reaktivierung einer Gedächtnisspur während dem Tiefschlaf diese neuronalen Verbindungen stärkt und gleichzeitig den Transfer von einem hippokampalen zu einem kortikalen Gedächtnissystem ermöglicht. Diese Konsolidierungsprozesse im Schlaf werden von unterschiedlichen oszillatorischen Ereignissen unterstützt. In dieser Dissertation werden vier Manuskripte, die die oszillatorischen Mechanismen der Gedächtniskonsolidierung untersuchen präsentiert.

Manuskript I zeigt, dass im Wachzustand anhaltende oszillatorische Veränderungen durch die gezielte Reaktivierung von Gedächtnisinhalten (targeted memory reactivation (TMR)) im Schlaf ausgelöst werden können. Insbesondere weisen Worte, die während dem Schlaf reaktiviert wurden bei einem Wiedererkennungstest erhöhte Thetaaktivität auf. Diese Aktivitätserhöhung tritt im linken inferioren Präfrontalkortex auf, einer Region, die mit vertiefter Wortverarbeitung assoziiert wird. Ausserdem unterstreicht die Studie die Wichtigkeit der Thetaaktivität bei gedächtnisbezogenen Prozessen im Gehirn.

Die Wichtigkeit des Zusammenspiels von langsamen Oszillationen (slow oscillations (SOs)) und Schlafspindeln wird in Manuskript II diskutiert. Zwei Verfahren, um mit auditorischen Stimuli die SOs zu verstärken, werden verglichen. Mit dem einen Verfahren kann die Gedächtniskonsolidierung verbessert werden, mit dem anderen nicht. Die Gedächtniskonsolidierung wird nur mit jenem Verfahren erreicht, dass auch Schlafspindeln induziert.

Manuscript III zeigt einen closed-loop TMR Ansatz, um auditorische Stimuli gezielt in die auf- oder absteigende Phase einer SO zu spielen. Das Abspielen von Wörtern in der aufsteigenden Phase verbessert die Gedächtniskonsolidierung gegenüber nicht eingespielten Worten. Das Einspielen in die absteigende Phase bringt nur eine leichte Verbesserung gegenüber uneingespielten Worten. Im Nachhinein korrekt erinnerte Worte, die in die aufsteigende Phase eingespielt wurden, weisen gegenüber nicht erinnerten Worten im Schlaf einen Anstieg in Spindel- und Thetaaktivität kurz nach Stimuluspräsentation auf. In die absteigende Phase gespielte Worte führen zu keinem oszillatorischen Unterschied.

Zusammenfassend, zeigt diese Dissertation die Wichtigkeit des Zusammenspiels von SOs, Schlafspindeln und Thetaaktivität auf. Ausserdem wird die Wichtigkeit des ‚Wann' und ‚Wie' der Gedächtniskonsolidierung bestätigt.

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1 Introduction

Over the last decades research has shown, sleep is not merely a passive state of reduced consciousness, but rather an active and important part of maintaining an organism's integrity, health and functionality (Mistlberger, 2005; Rasch & Born, 2013; Ringli & Huber, 2011; Van Cauter, Spiegel, Tasali, & Leproult, 2008; Wulff, Gatti, Wettstein, & Foster, 2010). In fact, humans spend about one third of their life in this unconscious and minimally reactive state. During sleep, the brain is decupled from external stimuli, which allows it to reorganize itself without interference. This suggests that the beneficial processes of sleep are related to the brain itself (Hobson, 2005). Although there has been no consensus on the core function of sleep, its universality across the whole animal kingdom, the tight regulation of sleep and the fact that it cannot be eliminated without deleterious consequences to the organism, indicates great importance for the role of sleep (Cirelli & Tononi, 2008).

As an additional key function of sleep, memory consolidation has been identified and researched extensively (Born, 2010; Born & Wilhelm, 2012; Crick & Mitchison, 1983; Diekelmann & Born, 2010; Marr, 1971; Rasch & Born, 2013; Tononi & Cirelli, 2006, 2014). Memory consolidation, in particular memory consolidation of declarative memory (see section 'Types of Memory') during sleep will be the focus of this thesis. Following in this chapter is a short overview of its mechanism and the embedding of the four manuscripts presented in the thesis.

The mechanism by which memory consolidation is achieved – it is thought to be the spontaneous reactivation of memory traces during sleep (Diekelmann & Born, 2010) – , will be elaborated in the section 'Active System Consolidation Hypothesis'. To enhance memory performance specifically, researchers have coupled memory traces to specific cues (e.g. auditory (Schreiner & Rasch, 2015a), olfactory (Rasch, Büchel, Gais, & Born, 2007) cues). This process is called targeted memory reactivation (TMR) and will be described in section 'Targeted Memory Reactivation'. In addition to reactivation of a neuronal memory trace, consolidation includes the reorganization of memory from a hippocampal short-term memory system to a neo-cortical long-term memory system (see sections 'Two-Stage Model' and 'Active System Consolidation Hypothesis' for elaborations). How is this transfer possible? Specific neural processes known to be crucial to memory consolidation can be measured with surface electroencephalogram (EEG) as oscillatory events (G. Buzsaki, 2004). Specifically, high amplitude and low frequency oscillations (see section 'Slow Wave Activity) have been suggested to facilitate the communication between different brain regions (Diekelmann & Born, 2010; Rasch & Born, 2013; Staresina et al., 2015). Other types of oscillations are involved in more fine grained timing (see section 'Sleep Spindles') or reflect access and manipulation of

memory traces on a neuronal level (see sections 'Sharp Wave Ripples' and 'Theta Activity'). The aim of this thesis is to expand the understanding of these oscillatory mechanisms and their interplay.

Following, is a brief description of the manuscripts presented in this thesis. A more thorough summary, including the addressed research questions, is given in the chapter 'Overview Manuscripts and Research Questions'.

Manuscript I shows theta activity (see section 'Theta Activity') to be a reliable indicator of successful memory recall during wake. Theta oscillations were previously not found to be related to NREM sleep. Only recently, preceding studies have found theta to be a marker for successful memory reactivation during sleep (Groch, Schreiner, Rasch, Huber, & Wilhelm, 2017; Lehmann, Schreiner, Seifritz, & Rasch, 2016; Schreiner, Lehmann, & Rasch, 2015; Schreiner & Rasch, 2015a). These results are confirmed in Manuscript III.

Manuscript II discusses the potential and limits of stimulating and increasing slow oscillations (SOs) during NREM sleep. The theory suggests that memory reactivation during sleep is optimal during certain time windows. These are the so-called up-states of slow oscillations (see section 'Slow Wave Activity). This has so far however not been confirmed experimentally in humans.

Therefore, manuscript III applies closed-loop (i.e. targeted stimulation through online EEG monitoring) TMR to target the up-states, windows of highly synchronized neuronal activity, of SOs specifically. The results show, SO up-states to be optimal for TMR. The importance of theta shown in manuscript I during wake, is also a crucial marker for indicating the success of memory reactivation during up-states.

Finally, manuscript IV describes an experiment to test a simple TMR setup at home and shows the feasibility of enhancing memory consolidation in an every-day setting. TMR has previously not been tested in an uncontrolled environment and over multiple days. This study is therefore able to highlight the challenges and crucial points to consider, when translating TMR from a lab setting to a scheme suitable for daily use.

After this short introduction, a theoretical section will introduce the relevant concepts of memory (chapter 'Memory'), sleep (chapter 'Sleep') and their functional interaction (section 'Sleep and Memory'). After the theoretical introduction the manuscripts and their research questions are summarized (see chapter 'Overview Manuscripts and Research Questions'). Then the four manuscripts, that show the original research, will be presented. The thesis will be concluded by a coherent discussion of the research results

and how they are connected. Additionally open research questions will be identified and possible explanatory models will be proposed. Finally, the two eminent theories concerning the function of sleep, the active system consolidation hypothesis and synaptic homeostasis hypothesis will be discussed in relation to the findings.

2 Theoretical Background

2.1 Memory

As laid down in the previous chapter, memory is the key component of this thesis. This chapter will introduce the different types of long-term memory (LTM). Of these, the presented studies (manuscripts) will all focus on declarative memory. It is not the aim of this thesis to discuss fully the different types of LTM. Rather it is to give a brief overview and circumscribe the type on which the studies of this thesis focus.

Next, a model for memory formation is outlined, which describes the different stages of memory formation (see section 'Multi Store Model'). The model shows that short-term memory (STM) is transferred into LTM through the process of reactivation. As shown in the 'Introduction' chapter, this thesis focuses on the consolidation of LTM.

The two-stage model introduced next (section 'Two-Stage Model') explains how LTM is organized in the brain. It proposes an organization of the LTM into a fast learning hippocampal system and a slow-learning neocortical system. The transfer of information between these two systems is thought to happen during sleep (Diekelmann & Born, 2010).

2.1.1 Types of Memory

There are different types of memories stored in the brain and while it is clear that the different memory types have distinct forms of processing and are potentially stored in different ways within the brain, these processes are not yet fully understood (Poldrack & Packard, 2003; L. R. Squire & Zola, 1996; Larry R. Squire, 2004; Tulving, 1972). The model of Squire and Zola (1996) gives a good overview over the different types of memories that are distinguished.

They present a taxonomy of the long-term memory systems, which will be briefly summarized below (see Figure 1). Long-term memory is typically subdivided into declarative (explicit) memory and non-declarative (implicit) memory (Roediger, 1990; L. R. Squire & Zola, 1996). A brief overview of both is given below.

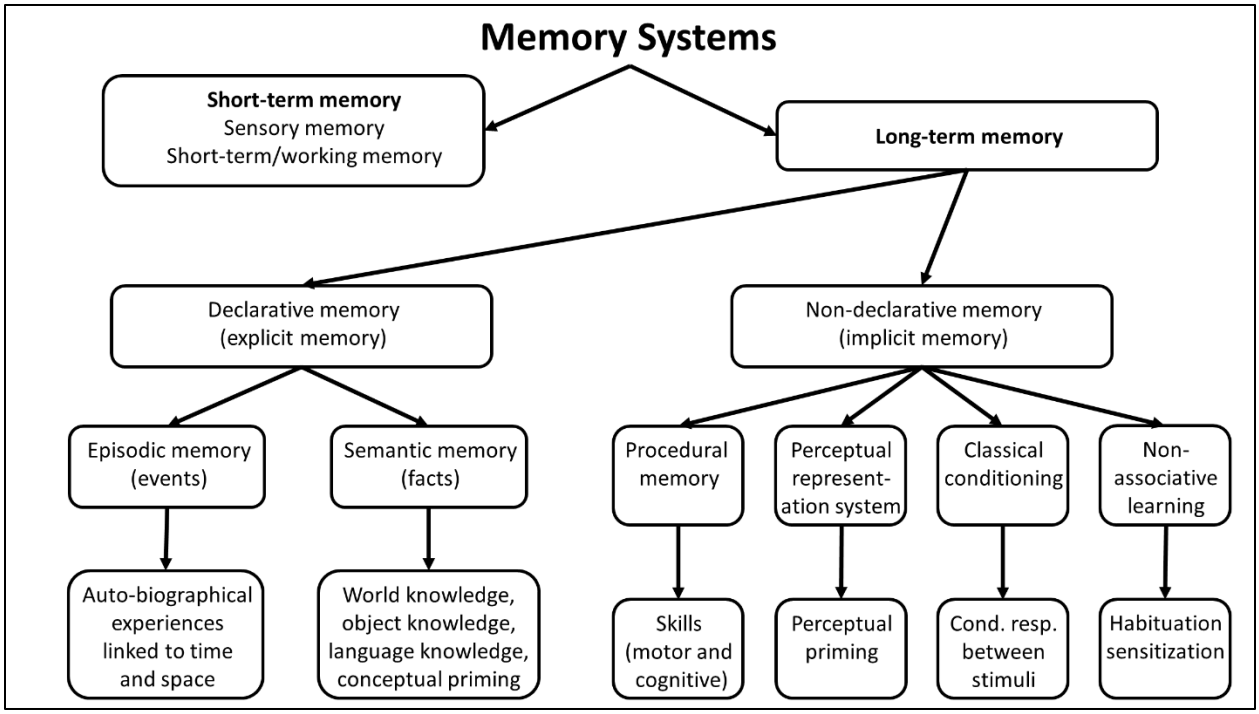

Figure 1| Different types of memory systems in the brain. Memories stored in different memory systems. Short-term memory includes sensory and working memory, which are represented in the brain for under a second and up to a few seconds respectively. Long-term memory is divided into declarative and non-declarative memory. Declarative (explicit) memory constitutes episodic (event) memory and semantic (fact) memory. Non-declarative memories constitute procedural memory, perceptual representation system, classical conditioning and non-associative learning (see text for further explanations). Figure adapted from (L. R. Squire & Zola, 1996).

Declarative memory:

Declarative memory is subdivided into two types: *episodic memory* and *semantic memory* (Tulving, 1972).

Episodic memory contains specific personal experiences linked to a particular temporal and spatial context, its retrieval changes its content and it is vulnerable to forgetting (Tulving, 1986; Wixted, 2004). *Semantic memory* on the other hand is focused on the knowledge of facts (Tulving, 1983). It does not register information with temporal and spatial context, its retrieval does not change the content and it is relatively stable (Tulving, 1972).

Non-declarative memory:

Non-declarative memory can be subdivided into *procedural memory*, *perceptual representation system*, *classical conditioning* and *nonassociative learning* (Henke, 2010; Schacter, 1987).

Procedural memories are motor or cognitive skills one has learned, such as riding a bike or playing the piano (Willingham, 1998). The *perceptual representation systems'* purpose is to rapidly identify formerly

encountered stimuli (Jacoby & Dallas, 1981). *Classical conditioning* links two unrelated stimuli together to learn a new response by association (Clark & Squire, 1998; Pavlov, 1927). *Non-associative learning* can be differentiated into habituation and sensitization (Kandel, 2001). The former describes the process of a decline in response to a repeatedly presented stimulus (H. M. Pinsker, Hening, Carew, & Kandel, 1973); the latter describes the reduction of the activation threshold of a behavioral response towards a relevant stimulus in response to its repeated presentation (H. Pinsker, Kupfermann, Castellucci, & Kandel, 1970).

All memory experiments discussed in this thesis test semantic memory; more specifically vocabulary learning: A German-Dutch foreign vocabulary-learning task is used in Manuscripts I, III and IV. Manuscript II discusses experiments using a paired-noun-associates task. While some concepts and ideas discussed in this thesis may be transferrable and generalized to other forms of memory, they are primarily discussed and studied in the context of semantic memory.

2.1.2 Multi Store Model

The multi store model by Atkinson and Shiffrin (1968) proposes consecutive memory systems to filter information and stabilize memories in the brain. There must be a path from the perception of the environment at the periphery of our sensory systems to the formation of an enduring LTM. In this simplified model, the memory process can be thought of as a storage system with multiple stages (Atkinson & Shiffrin, 1968; see Figure 2). Sensors register inputs from the environment, which enter the sensory memory system. This system is sense specific and can store large amounts of information for a very short time (< 0.5 seconds) (Sperling, 1960, 1963). If this information is attended to, it enters the STM, sometimes also referred to as the working memory, but see e.g. (Cowan, 2009; Engle, 2002) for distinctions. In STM, information that is currently needed for a cognitive task is rehearsed and updated (Baddeley, 2012). This memory system is limited to 7 ± 2 items and has a persistence of a few seconds (Miller, 1956). If items are not constantly rehearsed, they are forgotten through displacement or decay (Baddeley, 2003; Jonides et al., 2008). From STM, memories can be encoded into LTM (Baddeley, 2003). These memories are fragile and get more robust over time through the process of consolidation (McGaugh, 2000). The LTM system is practically unlimited in its capacity and can store memories up to a lifetime (McGaugh, 2000). To recall a memory, it is again retrieved from LTM into STM, where it can be accessed again and made available for further processing (Baddeley, 2012).

This model is an oversimplification and does not account for different types of memories, learning strategies, brain areas involved etc. It does however emphasize, that memory acquisition is divided into distinctive processing steps. The question remains however how these LTMs can be formed in the first place.

To address this, the following section proposes an LTM system that can rapidly encode memory traces during learning and later integrate them into more stable and long lasting memory representations.

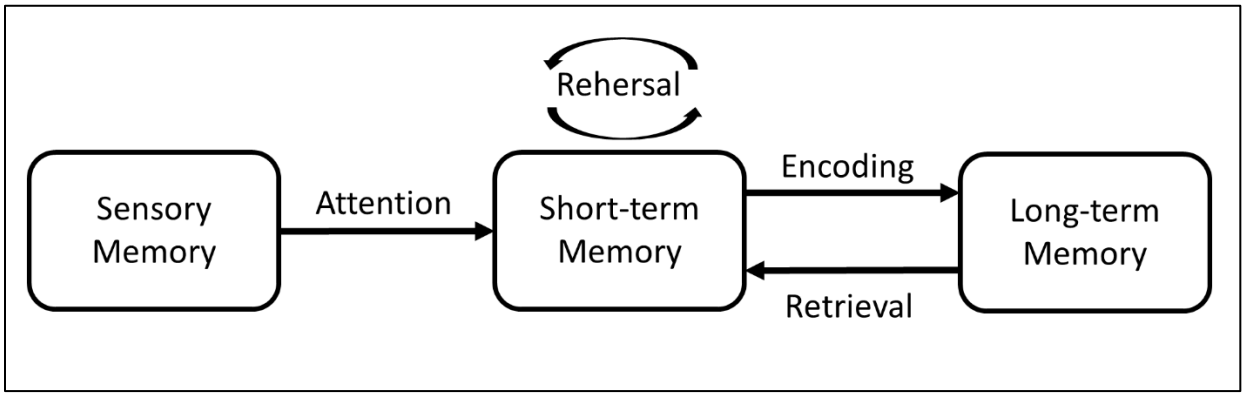

Figure 2| Schematic representation of the multi store model. Sensory information is acquired and can be stored in the sensory memory in large amounts but for only very short periods. If the information is attended it can move to the short-term memory. In this memory system, only few memories can be kept and they have to be continually rehearsed so as not to be lost. Through encoding memories can be transferred to the long-term memory system. This system is practically unlimited in its capacity and can potentially store memories for a lifetime. If a memory needs to be accessed again it can be retrieved from the long-term memory system and is brought back into the short-term memory system. Adapted from (Atkinson & Shiffrin, 1968).

2.1.3 Two-Stage Model

This thesis focuses on the LTM. Specifically the process of memory consolidation, where newly acquired memories initially stored in the hippocampus are gradually transferred into the neocortex. This process on the one hand allows for very long time memory storage and on the other hand for abstraction, generalization and the integration into pre-existing knowledge networks (Diekelmann & Born, 2010; Nere, Hashmi, Cirelli, & Tononi, 2013; Rasch & Born, 2013; Tononi & Cirelli, 2014). The two-stage model outlined in this section addresses this and is therefore a valuable addition to the memory formation model of Atkinson & Shiffrin (1968).

The two-stage model of memory (Marr, 1971; McClelland, McNaughton, & O'Reilly, 1995) states that effective learning requires two complementary systems. One for the rapid learning of specifics of individual items and experiences. The other serves as a storage of structured knowledge of the environment, acquired gradually over time through repeated exposure. In the brain, these two systems are namely the hippocampus and the neocortex (Kumaran, Hassabis, & McClelland, 2016; McClelland et al., 1995); see Figure 3). The hippocampus, with high synaptic plasticity, stores the initial inputs during wake learning. During the consolidation process, the initially stored memory traces are transferred through bidirectional connections between the hippocampus and the neo-cortex. At the same time, memory traces are integrated into existing neo-cortical knowledge networks.

Again, this model is a simplification and does not reflect the intricacies of the biological memory system. It does not offer a mechanistic explanation by which memories are transferred from the hippocampal to the neocortical system. In addition, it does not account for different types of memories and intermediary brain areas involved in the transfer. Nevertheless, the model captures the need for the interplay of a rapidly encoding and a slow acquiring memory system. The active system consolidation introduced in section 'Sleep and Memory' builds on this model and offers a mechanism for the transfer between the fast and slow LTM system.

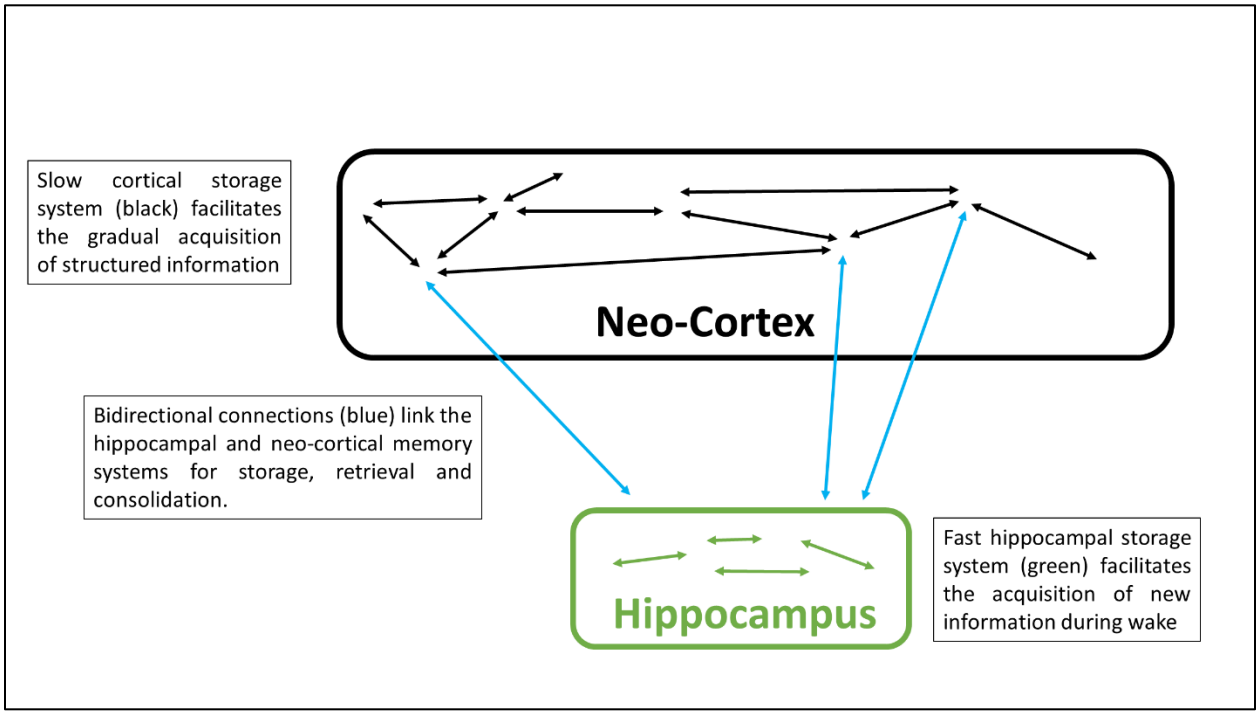

Figure 3|Schematic representation of the two-stage model of memory. Initial sensory inputs during wake learning are rapidly encoded in the hippocampus (green) with high synaptic plasticity. During the consolidation process, the initially stored memory traces (green arrows) are transferred through bidirectional connections (blue arrows) between the hippocampus and the neo-cortex (black). At the same time, memory traces are integrated into existing neo-cortical knowledge networks (black arrows), leading to systems-level consolidation. Image adapted from (Kumaran et al., 2016).

The process of transferring information from the hippocampus to the neocortex is termed consolidation (McGaugh, 2000). It turns out that this process mostly happens during sleep (Rasch & Born, 2013). The following chapter will therefore briefly introduce sleep and some of its main properties, followed by a chapter on sleep and memory processes, as they are relevant to the scope of this work.

2.2 Sleep

Historically sleep has been considered a passive state of inactivity for the brain (Datta, 2010). Only with the development of the EEG by Berger (1929) it became possible to measure the electrical activity of the brain during this state. It turns out that the brain is indeed active during sleep and it cycles through different sleep stages throughout the night (Iber, Ancoli-Israel, Chesson, & Quan, 2007). This chapter introduces the architecture of sleep with its different sleep stages. Followed by a description of prominent sleep-specific oscillatory events as they are relevant for the presented manuscripts.

2.2.1 Sleep Stages

Sleep stages are divided into N1, N2 and N3 sleep as well as REM sleep ((Iber et al., 2007); see Figure 4). N1 sleep is the transition from waking to a sleep state (Iber et al., 2007). Single high-amplitude slow waves as well as sleep spindles characterize N2 sleep (D.-J. Dijk, 2009). N3 sleep, also termed slow wave sleep (SWS), is dominated by slow oscillating, high amplitude waves (D. J. Dijk, Hayes, & Czeisler, 1993). N2 and N3 sleep are summarized as NREM sleep.

REM sleep is the stage most similar to waking in terms of EEG. Low overall amplitude and fast EEG activities are the hallmarks of REM sleep (McCarley, 2007). Additionally, characteristic ocular movements as measured by electrooculography (EOG) and low muscle tonus accompany it (Iber et al., 2007).

During sleep the different sleep stages alternate (Dement & Kleitman, 1957). The characteristic pattern are 90-minute blocks of N2, SWS and REM sleep, repeated 4 to 6 times per night in a healthy humans night sleep (Walker & Stickgold, 2004). While N2 sleep is the most prevalent sleep stage across the night (Ohayon, Carskadon, Guilleminault, & Vitiello, 2004), the proportions of SWS and REM sleep shift over the night (Walker & Stickgold, 2004). While the first cycles are dominated by SWS, it is hardly present towards the end of the sleep phase. REM sleep gradually takes over an increasing portion of the time within a cycle across the night (see Figure 4).

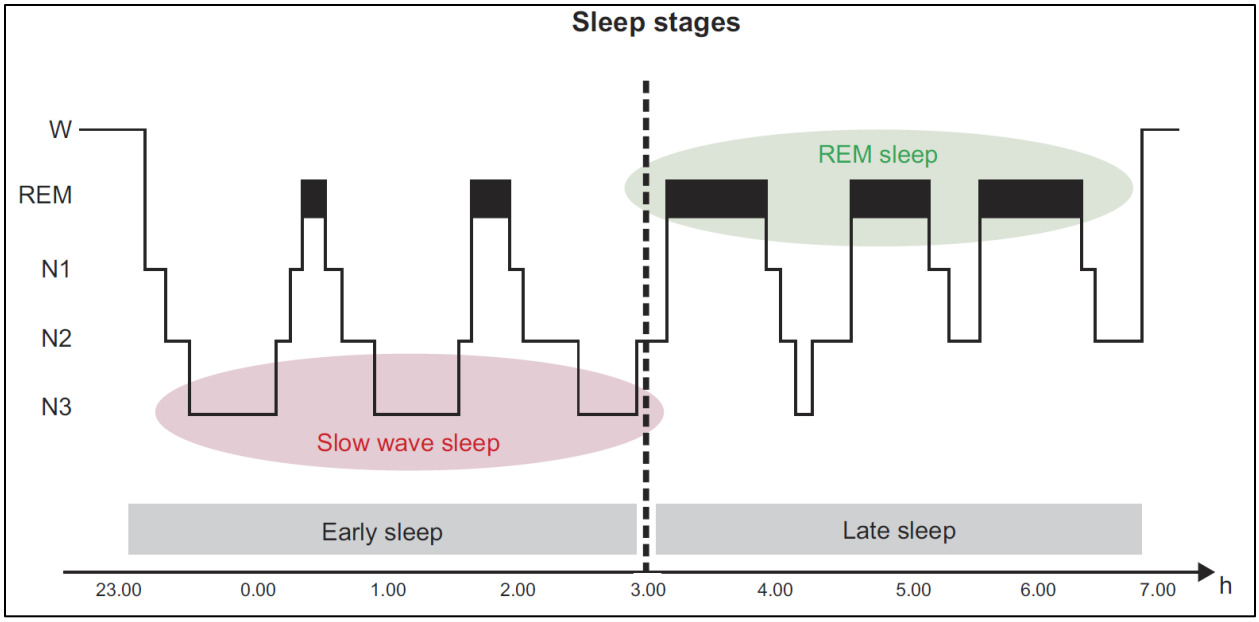

Figure 4| The cyclic pattern of sleep stages across a nights' sleep. 90-minute NREM-REM cycles repeat through the night. Early sleep is predominated by NREM sleep and late sleep by REM sleep. Adapted from (Rasch & Born, 2013).

For the consolidation for declarative memories, NREM (N2 and SWS combined) sleep has been shown to be crucial (Rasch & Born, 2013; Schreiner & Rasch, 2015a). The effect of REM sleep on consolidation remains elusive, however it is hypothesized that REM sleep stabilizes memory traces through synaptic consolidation that have been qualitatively reorganized during NREM system consolidation (Diekelmann & Born, 2010; Rasch & Born, 2013). The cyclic nature of NREM and REM sleep may hint at an interplay between these two stages (Diekelmann & Born, 2010; Giuditta et al., 1995; Rasch & Born, 2013). It has also been proposed that NREM sleep alone is capable of all required memory processing, though less efficiently than in conjunction with REM sleep (Kavanau, 2005). The mechanism by which NREM and REM sleep interact is however not fully understood and is an open research question. This thesis focuses on memory reactivation during NREM sleep. Nonetheless, we report a positive link between REM sleep and memory consolidation in manuscript III. Possible connections between NREM and REM sleep will be addressed in the discussion, but the mechanisms and further functions of REM sleep for memory consolidation are out of scope.

2.2.2 Sleep Specific Oscillations Relevant to Memory Processes

Specific neural processes known to be crucial to memory consolidation can be measured with surface EEG as oscillatory events (G. Buzsaki, 2004). These oscillations are understood to time and gate access to memory representations stored in the brain (Diekelmann & Born, 2010; Staresina et al., 2015). To under-

stand the process of memory consolidation it is therefore essential to understand the function and interdependencies of these oscillatory events. In the following sections, characteristic oscillatory events as they occur during sleep are introduced. First slow and high amplitude oscillation as they are present in N2 and SWS are described (see section 'Slow Wave Activity'). Next are sleep spindles (see section 'Sleep Spindles'), characteristic oscillatory events of N2 sleep, that co-occur with slow oscillations. Sharp wave ripples (see section 'Sharp Wave Ripples') are short bursts of activity nested insight spindle troughs. Surface EEG does not measure sharp waves, but they are associated with the activation of a single memory trace. These ripple events are described nonetheless, because they are integral to the active system consolidation theory (see section 'Active System Consolidation Hypothesis'), even though they cannot be measured in the studies presented in this thesis. The final section addresses theta oscillations (see section 'Theta Activity'). These are not considered to be classical representations of sleep oscillations in humans. Rather they have recently be found to be a marker of successful memory reactivation during TMR (Groch et al., 2017; Lehmann et al., 2016; Schreiner, Lehmann, et al., 2015; Schreiner & Rasch, 2015a). Theta activity during the memory consolidation process will also be addressed in manuscript III (Göldi, van Poppel, Rasch, & Schreiner, 2017).

2.2.3 Slow Wave Activity

Slow wave activity (SWA) is activity in the 0.5 to 4 Hz frequency band (as measured by scalp EEG). It can be divided into slow oscillations (SOs) and delta waves. The frequency range of 0.5 to 1 Hz makes up the SOs, with a peak frequency of 0.8 Hz (Achermann & Borbély, 1997). The 1 to 4 Hz frequency band is termed delta waves.

In addition to their low frequency, slow waves also have a large negative amplitude, typically exceeding 75 uV. The amplitude and slope of a slow wave is directly related to the synchrony of the neuronal activity, and therefore to the underlying degree of connection between these neurons (Esser, Hill, & Tononi, 2007; Olcese, Esser, & Tononi, 2010).

While it is not yet clear if there is a functional difference between SWA and SOs, mechanistically they are assumed to be generated by the same process. A major function of the slow oscillation is thought to be the widespread synchronization of cortical and thalamo-cortical networks (Rasch & Born, 2013). It provides a global clock where neuronal processing is limited to up-states following neuronal hyperpolarization (Compte et al., 2008; Destexhe, Hughes, Rudolph, & Crunelli, 2007; Fuentealba, Timofeev, & Steriade, 2004; Luczak, Bartho, Marguet, Buzsaki, & Harris, 2007; Mölle & Born, 2011; Mölle, Yeshenko, Marshall,

Sara, & Born, 2006; Rasch & Born, 2013; Steriade, McCormick, & Sejnowski, 1993). The manipulation (specifically induction) of slow waves is further discussed in manuscript II. The experiment presented in manuscript III will specifically target the up- and down-states of SOs with memory cues to test their functional relevance for memory consolidation.

SWA is predominant in SWS, where trains of multiple slow waves can be detected. During sleep stage N2, the slow waves are more likely to appear as single isolated high-(negative)amplitude waves often called K-complexes (Sydney S Cash et al., 2009). Whether isolated SOs are distinct events from K-complexes (KCs) is not clear and will be further elaborated in the discussion.

Sleep slow waves tend to originate in the medial prefrontal cortex and then propagate to the medial temporal lobe and hippocampus (Nir et al., 2011). They are generated by periods of highly synchronized neuronal depolarization with concomitant neuronal firing (up-states) and synchronized neuronal hyperpolarization with concomitant neuronal quiescence (down-states) (Contreras & Steriade, 1997; Steriade, 2006; Steriade, Contreras, Curró Dossi, & Nuñez, 1993; Steriade, McCormick, et al., 1993; Steriade, Timofeev, & Grenier, 2001; Timofeev, Grenier, & Steriade, 2001). The beginning of the neuronal down to up state transition coincides with the negative peak of the down-state as measured by the scalp EEG (Riedner, Hulse, Murphy, Ferrarelli, & Tononi, 2011; Volgushev, 2006). The neuronal up to down state transition correspondingly coincides with the up-state peak measured by scalp EEG (F. Amzica & Steriade, 1998; Vyazovskiy et al., 2009).

The strength of SWA is a function of sleep pressure (A A Borbély, 1982; Daan, Beersma, & Borbély, 1984). The longer the wake phase lasts, the stronger the need for sleep becomes. Consequently, there is more SWA during the ensuing sleep phase. The decrease of sleep pressure during sleep is in turn reflected in a decrease of SWA across the night (Alexander A. Borbély & Achermann, 1999; Riedner et al., 2007; Vyazovskiy, Riedner, Cirelli, & Tononi, 2007). These results have been interpreted to be indicative of a reduction in synaptic strength through synaptic homeostasis across the night (Bersagliere & Achermann, 2010; Esser et al., 2007; Riedner et al., 2007; Vyazovskiy et al., 2009, 2011, 2007). Additionally SWA in humans changes drastically across the lifetime, increasing in young children until the onset of puberty and then declining during adolescence, and decreasing more slowly in adulthood until old age reflecting the developmental changes of neuronal connectivity (Buchmann et al., 2011; Ringli & Huber, 2011). See also section ‘Active System Consolidation Hypothesis’.

2.2.4 Sleep Spindles

Sleep spindles are waxing and waning bursts (0.5 to 3 seconds) of activity in the range of 9 to 15 Hz that are prevalent during N2 sleep (De Gennaro & Ferrara, 2003). They are generated in the thalamic reticular nucleus (von Krosigk, Bal, & McCormick, 1993) and thalamo-cortical projections mediate the propagation into cortical regions (De Gennaro & Ferrara, 2003; Rasch & Born, 2013). Typically, there is a distinction made between two types of spindles.

Fast spindles (12 to 15 Hz) are located over the central and parietal cortex and slow spindles (9 to 12 Hz) located over the frontal cortex (Anderer et al., 2001; Andrillon et al., 2011; De Gennaro & Ferrara, 2003; Nir et al., 2011). Fast spindles are primarily locked to the down-to up transition of the SO, while slow spindles are typically grouped before the negative peak of the slow oscillation (Mölle, Bergmann, Marshall, & Born, 2011). Fast spindles are related to cortico-thalamic activation (Doran, 2003) and associated with increased activation in the hippocampus which associates them with hippocampus-dependent memory processes (Rasch & Born, 2013). Slow spindles on the other hand result from cortical-cortical activation (Doran, 2003). They have been associated with general cognitive abilities in children (Hoedlmoser et al., 2014), but their precise function remains elusive. Manuscript III presents evidence that fast spindles are markers for successful memory consolidation.

2.2.5 Sharp Wave Ripples

A sharp wave ripple (SWR) is a brief (~80 ms) burst of activity that is observed in the local field potential of the hippocampus (Buzsáki, 1986; Buzsáki, Lai-Wo S., & Vanderwolf, 1983). In rats it is observed at ~200 Hz (Gyorgy Buzsaki, Horvath, Urioste, Hetke, & Wise, 1992) and ~80-140Hz in humans (Axmacher, Elger, & Fell, 2008; Bragin, Engel, Wilson, Fried, & Buzsáki, 1999; Clemens et al., 2007, 2011). The depolarization of a large number of pyramidal cells in the CA1 of the hippocampus, triggered by a synchronous discharge of bursting CA3 pyramidal neurons, leads to the formation of SWRs (Buzsáki, 1986, 1989). These bursts of activity are found to coincide with the replay of memory traces during SWS (Cheng & Frank, 2008; Girardeau & Zugaro, 2011; Kudrimoti, Barnes, & McNaughton, 1999; Nakashiba, Buhl, McHugh, & Tonegawa, 2009; O'Neill, Senior, Allen, Huxter, & Csicsvari, 2008; Roumis & Frank, 2015; Wilson & McNaughton, 1994). However it remains unclear whether all replay events are accompanied by SWRs and vice versa (Buhry, Azizi, & Cheng, 2011). Note that SWRs are local events originating in the sub regions of the hippocampus and can therefore not be measured with surface EEG. While the studies presented in this thesis are not able to measure SWR activity, ripple events are introduced here nonetheless, as they

constitute an integral part of the neocortex-hippocampus dialog essential to memory consolidation (see more in section 'Active System Consolidation Hypothesis').

2.2.6 Theta Activity

During wake theta (4 to 8 Hz) is associated with synaptic plasticity and long-term potentiation and is therefore associated with the acquisition and encoding of new memories (Axmacher, Mormann, Fernández, Elger, & Fell, 2006; Huerta & Lisman, 1995; Hyman, Wyble, Goyal, Rossi, & Hasselmo, 2003). Additionally theta activity is associated with high gamma activity (> 40 Hz), which is relevant to plastic processes and neuronal encoding (Canolty et al., 2006; Csicsvari, Jamieson, Wise, & Buzsáki, 2003; Fries, 2009; Staudigl & Hanslmayr, 2013). It has also been shown that increased theta during memory encoding is a marker for encoding strength (Wolfgang Klimesch, 1999; Osipova et al., 2006). And again in memory retrieval, increased theta has been associated with improved memory retrieval (W. Klimesch et al., 2001; Nyhus & Curran, 2010), which potentially reflects the strength of a memory trace (W. Klimesch et al., 2006).

In rats, theta activity during sleep is most consistent during REM sleep (Buzsáki, 2002) and correlates with the occurrence of ponto-geniculo-occipital waves (Karashima, Nakao, Katayama, & Honda, 2005). It is generated in the CA1 region of the hippocampus (Buzsáki, 2002; Goutagny, Jackson, & Williams, 2009). In the human brain, theta activity has remained elusive and cannot be pinpointed so clearly (Cantero et al., 2003).

Theta activity has typically not been associated to NREM sleep stages, probably because it does not stand out on its own. Unlike for spindle events there is no specific wave form or duration associated with theta activity. Rather, theta activity stands out when segments of successful TMR are contrasted with unsuccessful TMR (Groch et al., 2017; Lehmann et al., 2016; Schreiner, Lehmann, et al., 2015; Schreiner & Rasch, 2015a). Recent studies have shown that theta activity during TMR is stronger for words that are later successfully retrieved (Lehmann et al., 2016; Schreiner, Lehmann, et al., 2015; Schreiner & Rasch, 2015a). These results have been successfully reproduced for words replayed during slow oscillation up-states in manuscript III (Göldi et al., 2017).

Schreiner and Rasch (2015) showed an increase of theta during TMR in NREM sleep for words that were later successfully recalled relative to those that were not. EEG was also recorded during the subsequent recall and recognition session during wake. In Manuscript I (Schreiner, Göldi, & Rasch, 2015) the role of theta in successful memory recognition in a foreign word learning task is presented. If theta activity is a

predictive marker of successful memory encoding, it stands to reason that theta activity can also be measured in other memory related events.

2.3 Sleep and Memory

Jenkins and Dallenbach (1924) were the first to show that sleep enhances memory performance. As the previous chapters have shown, memory consolidation is a process that needs to transfer encoded memories between distinct brain regions (Kumaran et al., 2016; Marr, 1971; McClelland et al., 1995). In the waking brain, different regions are communicating and new sensory inputs are processed constantly (Kavanau, 1997; Thompson, 1986; Ungerleider, 1995). In this state of the brain, it is difficult to transfer memories between brain areas without interference and corruption (Born, Rasch, & Gais, 2006; Marshall & Born, 2007; McClelland et al., 1995). Therefore, an offline period is needed, where brain regions can communicate and transfer information over long distances. Sleep offers a state where minimal external inputs are processed (Kavanau, 2005) and structural reorganization can take place (Diekelmann & Born, 2010; Rasch & Born, 2013). It is therefore an ideal state, in which memory consolidation can take place without interference. Sleep is structured into functionally distinct repeating sleep stages (Lesku, Roth, Amlaner, & Lima, 2006; Rasch & Born, 2013). Within those, nested sleep specific oscillations provide a temporal frame within which memories can be accessed and transferred across the brain (Staresina et al., 2015).

This section will introduce the two main theories of how memory consolidation during sleep might work: The active system consolidation hypothesis (ASH) (Born & Wilhelm, 2012; Diekelmann & Born, 2010; Rasch & Born, 2013) and the synaptic homeostasis hypothesis (SHY) (Tononi & Cirelli, 2003, 2006, 2014). They both build on the two-stage model of memory described in section 'Two-Stage Model', assuming that the fast-learning memory system, the hippocampus, encodes new sensory information during the day. The transfer into long-term storage, the neocortex, is then realized during sleep. They differ in their assumption of the mechanism by which they assume consolidation is realized in the brain. The following sections will give a short overview of ASH and SHY and highlight their differences. A discussion and comparison of the two theories will be given in the discussion of this thesis.

2.3.1 Active System Consolidation Hypothesis

The main pillar of the ASH is the assumption that memories are consolidated during sleep through active replay of memory traces (Rasch & Born, 2013). During SWS, memories stored in the hippocampal structure are redistributed to cortical long-term storage sites and integrated into preexisting memory traces (Born & Wilhelm, 2012). This sleep stage is predominated by SO events. SOs group spindle activity, which

in turn groups sharp wave ripples (SWR) (Staresina et al., 2015). The exact process however is not fully understood and manuscripts I – III presented in this thesis aim to contribute findings to fill this gap.

Following, is a brief overview of ASH and the involved neuronal oscillations: Neocortical SOs are thought to facilitate long-range communication, i.e. the transfer of information between hippocampal and cortical structures. Thalamo-cortical spindles timed to the transition of the SO down- to up-phase induce enduring plastic changes in cortical areas (Rasch & Born, 2013). SWRs that accompany reactivation of local memory traces in the hippocampus (Wilson & McNaughton, 1994) are embedded in the troughs of sleep spindles. In effect, there is a 2-way dialogue between the neocortex and hippocampal circuits (Mitra et al., 2016). Slow waves feedforward from the neocortex. The hippocampus talks back by replaying memories during SWR. The interaction and grouping of the different wave types provide time windows within which the transfer of information between brain areas can be achieved (see Figure 5).

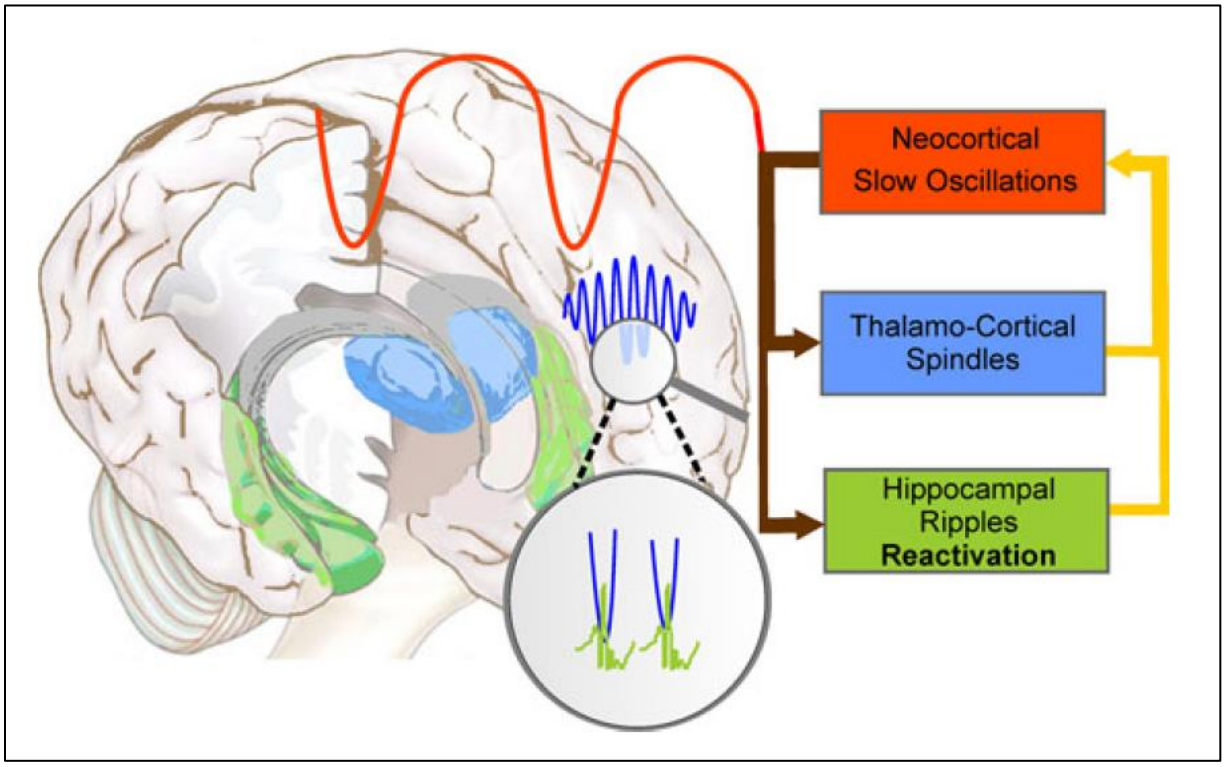

Figure 5| Schematic representation of the Active system consolidation hypothesis. Neocortical slow oscillations (red) provide a time window for the generation of thalamo-cortical spindles (blue). These in turn nest sharp-wave ripples (green) in their troughs. Ripple events accompany reactivation events of memory traces. These reactivations lead to a redistribution into existing memory networks within the neo-cortex. Figure adapted from (Born & Wilhelm, 2012).

The hierarchical nesting of oscillations serves as the theoretical motivation to target the slow oscillation up-state by closed-loop TMR as described in manuscript III. The functional relevance of NREM sleep for

memory consolidation also serves as a time frame reference for the practical study discussed in manuscript IV.

2.3.2 Synaptic Homeostasis Hypothesis

SHY proposes that the cellular homeostasis and the renormalization of synaptic strength is an important function of sleep (Tononi & Cirelli, 2006).The accumulation of synaptic strength through learning during the day is renormalized through a global down-scaling process during the brains offline period, i.e. sleep. Additionally it proposes that it is this synaptic downscaling of neurons that is beneficial for memory management (Tononi & Cirelli, 2014).

Computational models implementing this counter intuitive approach have shown it to work (Hill, Tononi, & Ghilardi, 2008; Nere et al., 2013) and explain not only the consolidation effect of sleep, but also gist extraction, integration and smart forgetting (Tononi & Cirelli, 2014). The driving mechanism of the neuronal downscaling is SWA. Through underlying postsynaptic processes (Czarnecki, Birtoli, & Ulrich, 2007) and spike time dependent plasticity mechanisms (Lubenov & Siapas, 2008), SWA directly drives the renormalization of synaptic strength. The benefit of synaptic downscaling comes from the improvement of the signal to noise ratio of encoded information (Hashmi, Nere, & Tononi, 2013; Nere et al., 2013). The key lies in activity-dependent downscaling of neurons (Hill et al., 2008; Olcese et al., 2010):

Neurons that are strongly connected and form a strong memory are more likely to fire together during SWA, which protects these connections from down-scaling. Random and weak connections (encoding noise) on the other hand do not fire together consistently and are therefore down-regulated (Nere et al., 2013). It is argued that the composition of neuromodulators during NREM favors synaptic depression (Tononi & Cirelli, 2014), but also see (Frank, 2012).

ASH and SHY propose two opposing mechanisms, i.e. active synaptic strengthening and global synaptic downscaling respectively, to explain memory consolidation. While at a first glance both hypotheses seem incompatible, they are both supported by a wealth of scientific evidence. While SHY is not part of the theoretical models underlying the argumentation in the manuscripts it is an eminent theory of the function of sleep and memory consolidation. It is therefore introduced here and will be further elaborated in the discussion of this thesis.

2.3.3 Targeted memory reactivation

The mechanism, by which memory consolidation is achieved, is thought to be the spontaneous reactivation of memory traces during sleep (see section 'Active System Consolidation Hypothesis' above). To enhance memory performance specifically, researchers (see below) have coupled memory traces to specific cues. These cues are then replayed during sleep with the goal to specifically reactivate the associated memory traces. This process is called targeted memory reactivation (TMR; see (Oudiette & Paller, 2013) for a review). TMR was experimentally shown for the first time in a seminal experiment by Rasch and colleagues (2007). They employed an object-location learning task in conjunction with olfactory stimuli. These stimuli where then administered again during sleep, resulting in an improvement of memory for stimuli-coupled memories. These results have since been replicated for contextual odor cues (Rihm, Diekelmann, Born, & Rasch, 2014; Suss, Gaylord, & Fagen, 2012) and specific item cues, such as sounds (Cairney, Lindsay, Sobczak, Paller, & Gaskell, 2016; Groch et al., 2017; Rudoy, Voss, Westerberg, & Paller, 2009), melodies (Antony, Gobel, O'Hare, Reber, & Paller, 2012; Cousins, El-Deredy, Parkes, Hennies, & Lewis, 2014; Schönauer, Geisler, & Gais, 2014) and verbal material (Lehmann et al., 2016; Schreiner, Lehmann, et al., 2015; Schreiner & Rasch, 2015a).

Manuscripts III and IV presented in this thesis also employ TMR to enhance memory performance. The aforementioned studies have found NREM sleep to be optimal for TMR to enhance memory consolidation of declarative memory. TMR during wake has shown no positive effect on memory performance (Schreiner & Rasch, 2015b), but TMR has been confirmed as a successful technique to enhance memory consolidation and the underlying mechanism is thought to be the active replay of memory traces (Diekelmann & Born, 2010; Rasch & Born, 2013; Rasch et al., 2007). This has been interpreted as support for ASH (Rasch et al., 2007), but computational models have shown the effects of TMR to also be compatible with the recent interpretation of SHY (Hashmi et al., 2013; Nere et al., 2013).

2.4 Overview Manuscripts and Research Questions

The manuscripts presented in this thesis are focused on understanding memory consolidation processes during sleep. First, manuscript I demonstrates that TMR during sleep not only changes memory outcome on a behavioral level, but also leaves persistent oscillatory changes in the waking brain. Manuscript II discusses the research in enhancing SWA with the goal of memory consolidation improvement. It highlights the importance of respecting endogenous brain rhythms during external stimulation. Manuscript III discusses the results of our research using closed-loop TMR. We find that the up-state of the SO is the optimal time window to reactivate memory traces. Manuscript IV describes the results of a first attempt

to utilize the successes of TMR of the past years to a simple and practical everyday home setting over multiple days. We find that TMR does not simply work 'out-of-the-box' and present evidence that TMR starts to show beneficial effects after an initial habituation period. The following sections will give a more thorough summary of the research questions and results for each manuscript.

Manuscript I (Schreiner, Göldi, et al., 2015)**:**

As shown in the section 'Theta Activity' above, neural oscillations in the theta band have been associated with successful memory encoding and retrieval during wake. Recent studies have also shown theta activity to be a marker for successful reactivation during sleep (Lehmann et al., 2016; Schreiner & Rasch, 2015a). However, it is unclear if successful reactivation of a memory trace during sleep has an effect on the oscillatory properties of the wake human brain at recall. We ask the questions: Does TMR during sleep induce observable oscillatory changes in the brain that persist in subsequent wake? And where are these changes located on source level?

In this study we find that cued words exhibit stronger centroparietal theta activity during word recognition compared to uncued words. Exploratory analysis shows that the increased theta activity originates in the left inferior prefrontal cortex. The same areas that are associated with increased neural activity in semantic (deep) versus perceptual (shallow) encoding (J. D. E. Gabrieli et al., 1996; Kapur et al., 1994), semantic versus phonological word processing (J. D. Gabrieli, Poldrack, & Desmond, 1998) and during word testing versus restudy (Van den Broek, Takashima, Segers, Fernández, & Verhoeven, 2013). Additionally, this study shows there is not only a behavioral outcome change on memory performance due to TMR. There is also an underlying neuronal oscillatory change that persists during wake after TMR.

Manuscript II (Göldi & Schreiner, 2017)**:**

Manuscript II (Göldi & Schreiner, 2017) is a commentary on (Weigenand, Mölle, Werner, Martinetz, & Marshall, 2016). We ask the questions: What are the differences between open- and closed-loop stimulation? How do these differences lead to different outcomes in memory consolidation? If SOs are not a sufficient marker for successful memory reactivation, what else is needed?

Given the assumed role of SOs in mediating the most prominent effects of sleep on cognitive functioning, several attempts have been made to potentiate them (Bellesi, Riedner, Garcia-Molina, Cirelli, & Tononi, 2014). While Ngo and colleagues (Ngo, Martinetz, Born, & Mölle, 2013) find a memory improvement effect when doing closed-loop auditory stimulation to enhance SOs, Weigenand and colleagues (Weigenand

et al., 2016) find no improvement in overnight consolidation of word pairs when SOs are enhanced through open-loop auditory stimulation during sleep.

The main difference between the two forms of stimulation is that during closed-loop stimulation, auditory clicks are played at the up-state peak of endogenous SOs which in turn elicits following SOs. During the open-loop stimulation, a first auditory click is used to induce a SO which is then targeted at its up-state peak to elicit further SOs. While SO power is enhanced in both paradigms, spindle activity is only enhanced during closed-loop stimulation. Considering it is specifically the nested interplay of SOs, sleep spindles and sharp wave ripples (see section 'Active System Consolidation Hypothesis') that enhance memory consolidation, this might explain the different behavioral outcome. In summary, this manuscript highlights i) the importance of the endogenous nature of neuronal rhythms and ii) the importance of the nested nature of neural oscillations in the brain, for them to be functionally significant.

Manuscript III (Göldi et al., 2017)**,** (submitted to eLife):

Manuscript III (Göldi et al., 2017) builds on the work presented in (Schreiner & Rasch, 2015a) and (Schreiner, Lehmann, et al., 2015) and builds on the insights gained in manuscript II (Göldi & Schreiner, 2017) to present a closed-loop TMR approach. We ask the questions: Can we improve the effectiveness of TMR? When is the optimal time window within an SO to play a memory cue? What is the oscillatory signature of a successful memory reactivation?

Word cues are targeted into the up- and down-state of SOs, in a within-subject design. Presenting memory cues during SO up-states robustly improves recall performance compared to uncued words. Presenting memory cues during SO down-states did not result in a clear behavioral benefit. Qualitatively down-state cued words showed a performance between that of up-state cued and uncued words. On a neural basis, successful memory reactivation during SO up-states was associated with a characteristic power increase in the theta and sleep spindle band. No oscillatory changes were observable for down-state cues.

The findings presented in this manuscript provide experimental support for the assumption that slow oscillatory up-states represent privileged time windows for memory reactivation. Additionally, the supposition that the interplay of SOs, theta and sleep spindle activity promote successful memory consolidation during sleep is supported.

Manuscript IV:

Targeted memory reactivation can improve memory performance in a foreign vocabulary-learning task. This has been shown in previous studies in the lab (Göldi et al., 2017; Schreiner, Lehmann, et al., 2015; Schreiner & Rasch, 2015a). However, can this procedure be translated to an every-day setting? Will TMR have beneficial effects in an uncontrolled environment where sleep is not constantly monitored and cues cannot be actively targeted into the desired sleep stages? In addition, what is the effect of cueing applied across multiple days?

Manuscript IV aims to address these questions for the first time. Participants learn and recall Dutch-German word pairs on four consecutive days. During the nights in between the Dutch words are cued for 1 hour using an mp3-player. Cueing starts 30 minutes after participants go to bed ready to sleep at home. Surprisingly we find no beneficial memory effect for cued words compared to uncued words. Only during the third night, there is a positive effect of cueing on memory performance. Indeed, it is only the poor learners (bottom third) that profit from the reactivation procedure. The results suggest that the unfamiliarity of the TMR procedure interferes with the consolidation effect and only after subjective sleep quality is restored to pre-experimental levels during the third night, consolidation benefits from TMR.

Manuscript IV shows that the transfer of TMR from lab to field is not as straight forward as one would hope. Being the first study to conduct TMR-research in the field it has highlighted potential pitfalls and areas that need to be further studied. For example, the need to confirm the effect of sleep quality with objective sleep quality measurements, the need to target specific sleep stages and the need to find a comfortable and practical cue presentation method.

3 Manuscripts

3.1 Manuscript I

Cueing vocabulary during sleep increases theta activity during later recognition testing

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Candidate contribution:

Maurice Göldi did the source analysis and wrote the corresponding sections of the manuscript.

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Abstract

Neural oscillations in the theta band have repeatedly been implicated in successful memory encoding and retrieval. Several recent studies have shown that memory retrieval can be facilitated by reactivating memories during their consolidation during sleep. However, it is still unknown whether reactivation during sleep also enhances subsequent retrieval-related neural oscillations.

We have recently demonstrated that foreign vocabulary cues presented during sleep improve later recall of the associated translations. Here we examined the effect of cueing foreign vocabulary during sleep on oscillatory activity during subsequent recognition testing after sleep. We show that those words, which were replayed during sleep after learning (cued words), elicited stronger centro - parietal theta activity during recognition as compared to non-cued words. The reactivation-induced increase in theta oscillations during later recognition testing might reflect a strengthening of individual memory traces and the integration of the newly learned words into mental lexicon by cueing during sleep.

Descriptors: theta, recognition memory, memory reactivation, sleep, EEG

Extensive evidence has accumulated that memory formation and consolidation during wakefulness and sleep are heavily based on neural oscillatory synchronisation (Diekelmann & Born, 2010; Fell & Axmacher, 2011). During wakefulness, especially oscillatory theta and gamma activity have been consistently linked to the encoding and retrieval of new declarative information (Nyhus & Curran, 2010). Theta and gamma activity seems to be related to long term potentation and synaptic plasticity, thereby facilitating the encoding of new memories (Axmacher, Mormann, Fernández, Elger, & Fell, 2006; Huerta & Lisman, 1995; Hyman, Wyble, Goyal, Rossi, & Hasselmo, 2003). Concerning the retrieval of declarative memories oscillatory theta is assumed to drive the hippocampus-dependent reinstatement of individual memories in parietal cortex, while gamma seems to bind contextual information into episodic representations (Nyhus & Curran, 2010). In line with this assumption several studies focusing on recognition memory, reported enhanced parietal theta power in association with correctly identified studied (old) words when compared with correctly rejected non-studied (new) words (Jacobs, Hwang, Curran, & Kahana, 2006; Kim et al., 2012) as well as enhanced theta – gamma coupling (E. Düzel et al., 2003; Emrah Düzel, Neufang, & Heinze, 2005). Furthermore with regards to word learning theta activity has been demonstrated to reflect lexical access and thereby the integration of new words in the existing mental lexicon (Bakker, Takashima, Hell, Janzen, & McQueen, 2015). Taken together, parietal theta activity is assumed to reflect the strength of episodic memory traces (Klimesch et al., 2006).

After their encoding, memories are consolidated during subsequent sleep, and it assumed that beneficial effect of sleep on memory is due to spontaneously occurring hippocampal memory reactivations during Non rapid-eye movement (Non - REM) sleep (Oudiette & Paller, 2013; Rasch & Born, 2013). A causal role of those memory reactivations is supported by studies showing that experimentally inducing reactivations during NonREM sleep by using associated memory cues benefits memory consolidation and activates hippocampal brain regions during sleep (Oudiette & Paller, 2013; Rasch, Büchel, Gais, & Born, 2007; Rudoy, Voss, Westerberg, & Paller, 2009; Schönauer, Geisler, & Gais, 2013; van Dongen et al., 2012). In spite of the strong evidence for a behavioral effect of cue during sleep on later memory retrieval, it is largely unknown whether cueing also affects retrieval-related brain responses after sleep.

In a very recent study, we have shown that a replay of prior learned Dutch words during sleep enhances the recall of the German translations of those words (Schreiner & Rasch, 2014). EEG was further recorded during recognition testing after sleep. We hypothesize that replaying Dutch words during sleep also increases theta oscillations during recognition, indicating a strengthening of memory traces and the lexical integration of the new words by cueing during sleep on a neural level.

Method

Participants

The data were taken from Schreiner & Rasch (2014). Detailed information about participants, stimuli, task, data acquisition sleep data and behavioral results can be found in Schreiner & Rasch (2014).

30 healthy, right-handed subjects (15 female) with German mother tongue and without Dutch language skills participated in two experimental groups. Data of two subjects had to be discarded due to technical problems, resulting in 14 subjects in the reactivation group and 14 subject in the control group. The study was approved by the ethics committee of the Department of Psychology, University of Zurich, and all subjects gave written informed consent prior to participating.

Material and Procedure

The learning phase started at 22.00h. Participants were confronted with 120 Dutch German word pairs. After completing the learning task, participants slept for 3 hours. In the reactivation group, 60 out of the 120 Dutch words learned before were replayed again via loudspeaker (50 dB) during Non - REM sleep. No words were replayed during sleep in the control group. All participants were awakened at ~ 2.15h and recall of the vocabulary was tested afterwards. In the recognition phase the 120 Dutch words included in the pre-retention learning list were presented again aurally intermixed with 60 entirely new Dutch words. After listening to each word participants had to indicate whether the word was old (part of the learning material) or new. If the current word was recognized as old, they were asked to give the German translation.

EEG recording and analysis

EEG was recorded using a high-density 128-channel Geodesic Sensor Net (Electrical Geodesics, Eugene, OR, USA). Impedances were kept below 50 kΩ. Voltage was sampled at 500 Hz and initially referenced to Cz. Offline EEG pre-processing was realized using Brain Vision Analyzer software (version: 2.0; Brain Products, Gilching, Germany). Data were re-referenced to averaged mastoids, low-pass filtered at 100 Hz and high-pass filtered at 0.1 Hz. Line noise contamination was removed by a 50 Hz notch filter. Epochs of 3,000ms (1,000ms baseline) were extracted. Trials were classified as correct rejections (correctly identified new words) and hits (correctly identified old words) and subsequently as cued and uncued hits (correctly identified old words replayed respectively not replayed during sleep). An interval ranging from -700ms to -100ms preceding stimulus onset served as baseline. Baseline correction was applied by subtracting the mean power of the baseline interval from every trial.

Artifact-affected trials meeting the following criteria were labeled and finally rejected: voltage values exceeding +/-75µV or a voltage drift of more than 75 µV. Eye blinks and movements were corrected using independent component analysis (Jung et al., 1998). Components and corresponding topographies associated with eye blinks and movements were detected visually and removed, with the performing investigator being blind to the condition. All succeeding analysis steps were realized with MATLAB (the MathWorks) using the open-source Fieldtrip toolbox (Oostenveld, Fries, Maris, & Schoffelen, 2011). In a first step the segmented data were averaged (evoked response) and subtracted from each trial. Afterwards the time-frequency analysis was computed for each trial. The evoked response was subtracted in order to reveal the induced power for each frequency band (Klimesch, Russegger, Doppelmayr, & Pachinger, 1998). The rationale behind this approach is that induced oscillations are generated by distinct high-order process (Singer & Gray, 1995) and are involved in binding distributed cortical representations (Guderian & Düzel, 2005). Time-frequency power representations were computed by using a Morlet wavelet approach. A sliding window with a step size of 50 ms was applied across the entire length of the epochs. Data were analyzed separately for low frequency range (2 – 40 Hz with a 1 Hz step size and a width of 7 cycles) and high frequency range (40 – 100 Hz with a 5 Hz step size and a width of 20 cycles). Frequencies of interest included theta, alpha, lower beta, higher beta and gamma. Selected theta frequencies (5 - 6 Hz) corresponded to a spectral bandwidth of 4.3 – 6.8 Hz, alpha (9 - 11 Hz) to 7.8 – 12.5 Hz, lower beta (14 Hz) to 12 – 16 Hz, higher beta (19 - 26) to 16.3 – 29.7 and gamma (60 – 90 Hz) to 57 – 95 Hz. All presented data represent absolute changes in power with regards to the baseline (− 700 to − 100 ms pre-stimulus onset). Source localization and its statistical analysis were done with the open-source toolbox SPM12 (http://www.fil.ion.ucl.ac.uk/spm). Starting with the artifact-rejected data the EEG signal was average referenced across all channels. The template T1-weighted MRI scan provided by SPM was used to create the head mesh compartmentalizing the head into different areas for all subjects. A cortical mesh with 8196 vertices, corresponding to the possible dipoles within the brain, was created. The template electrode positions for the 128-channel Geodesic Sensor Net EEG system were coregistered to the structural MRI in MNI space using the nasion and the left and right pre-auricular fiducials as landmarks. The forward model represents the effect each dipole in the cortical mesh has on the electrodes. It was computed using a 'EEG BEM' head model. The inverse reconstruction, which maps the sensor level data back to the dipoles inside the brain, was computed using the Parametric Empirical Bayesian approach (Phillips, Mattout, Rugg, Maquet, & Friston, 2005). The multiple sparse priors algorithm (Friston et al., 2008) with the standard SPM settings and using group inversion (Litvak & Friston, 2008) was chosen to compute the inversion. Theta activity (5-6 Hz) was then isolated in the time window where it's activity was enhanced in sensor

space (see results section for contrasts and their corresponding time windows). The resulting activation in source space was then converted into a 3D NifTI image for further statistical analysis in SPM.

Statistical Analysis

A nonparametric randomization test using cluster correction was applied for statistical analysis (Maris & Oostenveld, 2007). This approach controls the type I error rate with regards to multiple comparisons by clustering neighboring sensor pairs that show the same effect. For all included frequency bins, t-statistics were computed for all sensors and for each 50ms time. For all frequency bins in a given frequency band, t-statistics were computed for every channel and for each 50ms time bin within each segment. Clusters of contiguous sensors across participants with a threshold below a *p*-value of 0.05 (two sided) were identified. The cluster-level statistics was defined from the sum of the t-values of the sensors in a given cluster. The cluster with the maximum sum was used as the test statistics. The type I error rate for the complete set of 114 channels was controlled by evaluating the cluster-level test statistic under the randomization null distribution of the maximum cluster-level test statistic. This was obtained by randomizing the data between conditions across multiple subjects calculating *t* statistics for the new set of clusters. A reference distribution of cluster-level *t* statistics was created from 500 randomizations. The *p* value was estimated according to the proportion of the randomization null distribution exceeding the observed maximum cluster-level test statistic (Monte Carlo *p* value). A paired t-test was applied to the theta activation data in source space. Here, a threshold of $p = 0.005$ (uncorrected for multiple comparisons) with a minimal cluster size $k > 50$ voxels was applied for an exploratory data analysis.

Results

Behavioral Results

As reported previously, cueing during sleep increased cued recall of the associated German translation of the Dutch words after sleep (see Schreiner & Rasch, 2014). In contrast, recognition performance of the Dutch words did not differ between cued and uncued words ($p > 0.8$; for an overview of the behavioural results see Table 1).

Table 1. Overview of memory performance

		Cued	Uncued	*t*	*p*
Reactivation group					
Cued recall	*Learning*	29.87 ± 0.09	33.20 ± 2.54	-1.29	0.22
	Retrieval	31.40 ± 0.16	31.33 ± 2.17	0.04	0.97
	Change	+1.53 ± 0.79	-1.87 ± 0.70	3.52	0.003**
	% Change	105.15 ± 2.64	95.43 ± 2.07	3.43	0.004**
Recognition	*Hits*	52.40 ± 0.98	51.20 ± 1.57	1.33	0.80
	% Hits	87.33 ± 1.62	85.33 ± 2.62		
	d'	2.32 ± 0.15	2.32 ± 0.17	0.00	0.99
Control group					
Cued recall	*Learning*	30	31.93 ± 1.84	-1.04	0.31
	Retrieval	28.07 ± 0.71	29.27 ± 1.66	-0.77	0.45
	Change	-1.93 ± 0.71	-2.66 ± 0.89	0.79	0.44
	% Change	93.55 ± 2.37	92.80 ± 3.10	0.24	0.81
Recognition	*Hits*	50 ± 1.24	50.60 ± 1.55	-0.64	0.53
	% Hits	83.33 ± 2.07	84.33 ± 2.59		
	d'	2.01 ± 0.13	2.09 ± 0.16	-0.93	0.36

Data are means ± s.e.m; Numbers indicate absolute or relative values of correctly recalled or recognized words that where presented during sleep (cued words, 60 in total) or not (uncued words, 60 in total). For cued recall testing, number of correctly recalled words during the learning phase before and the retrieval phase after the retention interval are indicated. Change (% Change) refers to the absolute (relative) difference in performance between learning and retrieval phases. Hits (%Hits) refers to the absolute (relative) number of correctly recognized words as "old" (since %Hits = Hits*100/60, statistics are redundant). The sensitivity measure d' reflects recognition performance according to signal detection theory based on the proportion of Hits and False Alarms (Macmillan & Creelman, 2005) . *$p < 0.05$; ** $p < 0.01$.

Oscillatory results

In a first step, we examined the impact of successful recognition on oscillatory activity in the frequency bands of interest (theta, alpha, lower beta, higher beta and gamma) in both experimental groups. Thus we compared induced oscillatory power associated with correctly identified old and new words, irrespective of previous replay. Oscillatory activity with regards to old words in the alpha, the low beta range tended to exhibit power decreases, but these effects did not survive the cluster correction for multiple comparisons. Similarly, there were no significant differences with regards to old and new words in the high beta range. In contrast, induced theta and gamma power differed significantly between conditions in both experimental groups. The cluster-based statistical analysis revealed that in both experimental groups recognition of old words was associated with enhanced theta power in centro-parietal regions (both $p < 0.025$; time range reactivation group: 600 – 1,300ms; time range control group: 400 – 1,300ms, for illustration see Figure 1 and Supplementary Figure 3 for individual cluster maps). Furthermore old

words were associated with an enhanced gamma activity in a late time window over parieto-occipital regions (both $p < 0.025$; time range reactivation group: 1,000 – 1,600ms; time range control group: 1400 – 1,600ms). Theta power in source space showed significant differences between old and new words ($p < 0.005$ uncorrected, k > 50, time window 600 – 1,300ms) for the reactivation group ([-50 22 -2], t = 3.42, k = 98). The activity was located in the left inferior prefrontal cortex located in Brodmann areas BA 47 and BA45 (Broca's area, see Figure. 2).

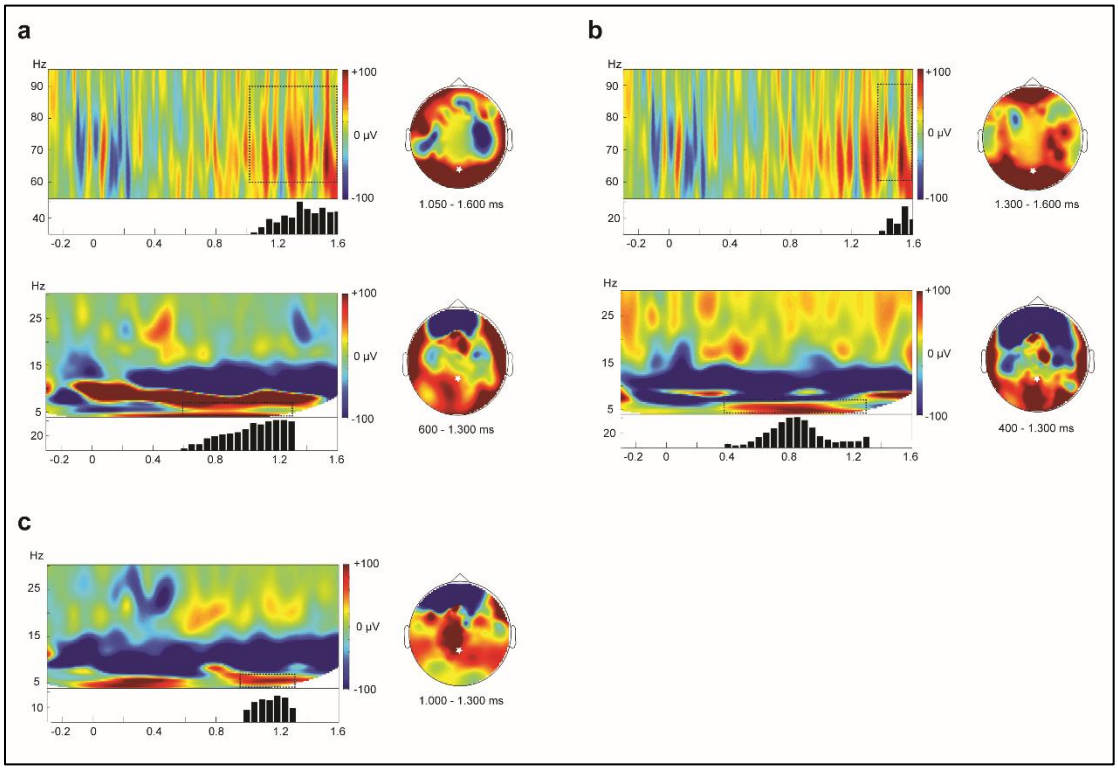

Figure 1| **Oscillatory results**. (a + b) Oscillatory activity recorded during recognition was computed for hits (words correctly identified as old) and correct rejections (words correctly identified as new). Gamma (upper panel, electrode Oz) as well as theta power (lower panel, electrode Pz) were significantly stronger in both, the reactivation (a) and the control group (b). Bar charts indicate the number of significant electrodes over time. (c) To specifically determine potential effects of cueing in the reactivation group the total number of recognized old words was divided into old words, which were replayed during sleep (cued) and old words not replayed during sleep (uncued). Cued old words elicited significantly stronger theta activity over centro-parietal regions.

To specifically determine potential effects of cueing on theta and gamma power in the reactivation group we further divided the total number of recognized old words into old words, which were replayed during sleep (cued) and old words not replayed during sleep (uncued). While no difference for cued and uncued old words appeared in the gamma range, induced centro-parietal theta power differed significantly between conditions ($p < 0.025$; time range: 1,000 – 1,300ms). In source space, however, the power difference did not reveal a significant difference at the exploratory significance level chosen here ($p < 0.005$ uncorrected, k > 50, time window 1000 – 1,300ms). To assure that this obtained effect is specific to cueing

during sleep, we divided the recognized old words of the control group also into the categories cued and uncued and analyzed induced theta activity for these two conditions. Please note that this separation was conducted artificially since in the control group no words were actually replayed during sleep. As expected no difference became apparent when comparing theta activity for cued and uncued old words in the control group, thereby strengthening the notion that our findings in the reactivation group were specific to cueing during sleep. Thus, our main analysis demonstrated that theta and gamma activity exhibited enhanced activity for old as compared to new words, while theta power was specifically affected by cueing during sleep.

In an additional step we investigated the influence of initial encoding strength on theta activity during recognition testing in the reactivation group. Therefore we divided all old words based on their recall success during prior encoding before sleep. Interestingly, theta activity was significantly stronger during recognition testing for those words, which had been successfully encoded before sleep ($p < 0.025$; time range: 650 – 1,000ms; for illustration please see Supplementary Figure 1 and 2), representing another hint that theta activity is associated with memory strength. Again, in source space, no significant activation was observed (time window 650 – 1,000 ms.) No difference in theta activity with regards to preceding encoding success emerged between cued and uncued words.

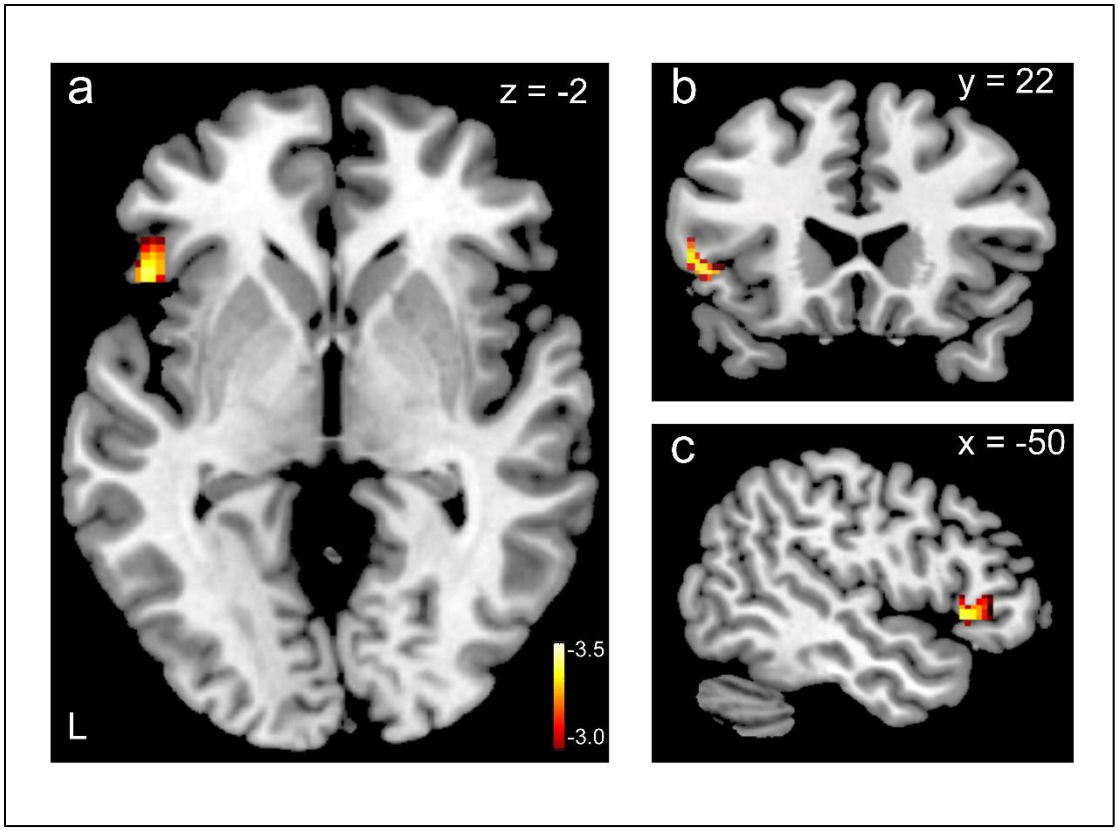

Figure 2| Old vs. New source estimates in the theta range (5-6 Hz). Theta power showed significant differences between old and new words in source space for the reactivation group (time window: 600 – 1,300ms). Activation was found in the left inferior prefrontal cortex, specifically Brodmann areas (BA) 47 and 45 (Broca's area). Activation is displayed on an anatomical template image on an axial (a), coronal (b) and sagittal (c) slice at a threshold of p = 0.005 and cluster size of k > 50.

To further assess potential influences of item characteristics on the obtained theta results we investigated whether the contiguity between the Dutch-German word pairs had an impact on our findings. Therefor we determined for every word pair the so-called Levensthein distance (e.g. Suárez, Tan, Yap, & Goh, 2011). The normalized Levensthein Distance ranges from 0 to 1 between phonetic forms of the words and represents a measure of concordance (0 = total concordance, 1 = no concordance). We divided old words in the two categories close (normalized Levensthein Distance < 0.5, 64 words) and far (normalized Levensthein Distance >0.5, 56 words) with regard to their German translations. There was no difference in theta activity between these two word categories, while a behavioral analysis revealed that close words, according to the Levensthein Distance, where significantly better recognized as compared to distant words (close: 91.2 % ± 1.7; distant: 81.5 % ± 2.8; $p < 0.001$). The interaction between Levensthein distance (close vs. distant words) and word reactivation (cue vs. uncued) was not significant ($p > 0.4$). Finally, we investigated whether the obtained effects are specific for recognition memory processes or whether theta activity during recognition might predict the likelihood for successful retrieval during subsequent cued recall

testing. Therefore we divided old words into Dutch words whose German translation was subsequently retrieved and those whose translation was not retrieved. No difference in theta activity became apparent when comparing these two categories.

Discussion

Our results demonstrate that cueing prior learned foreign vocabulary during sleep leads to enhanced theta activity in a subsequent recognition memory task. Theta activity as been repeatedly shown to be enhanced during successful memory retrieval (Nyhus & Curran, 2010), particularly in parietal regions (Jacobs et al., 2006) and might reflect the strength of a memory trace (Klimesch et al., 2006) and the integration of new words into pre-existing lexical networks (Bakker et al., 2015). According to the activity system consolidation theory, spontaneous hippocampal memory reactivations during SWS critically contribute to the memory-strengthening effect of sleep after learning. Several recent studies have successfully used memory-associated odors, sounds, or vocabulary (Rasch et al., 2007; Rudoy et al., 2009; Schreiner & Rasch, 2014) to target reactivation during sleep and thereby improve retrieval performance on the behavioral level. Our results add to this support by showing that targeted memory reactivation during sleep also increases oscillatory neural markers in the theta-band in parietal brain areas, possibly indicating an increase in memory strength and lexical integration by cueing during sleep. Interestingly, given that cueing during sleep behaviorally only exerted beneficial effects on memory performance acquired by cued recall but not on recognition memory, theta activity associated with recognition memory processes seems to represent a more fine-grained measure to index memory strength. Previous studies (J. D. E. Gabrieli et al., 1996; Kapur et al., 1994) have shown that semantic ('deep') versus perceptual ('shallow') word encoding leads to increased neural activation in Brodmann areas 45, 46, 47 of the left inferior prefrontal cortex. Within these regions, we observed increased theta-band activity associated with previously learned words versus new words during recall using a source analysis approach. Further fMRI studies have found higher activations in these regions in semantic versus phonological word processing (J. D. Gabrieli, Poldrack, & Desmond, 1998) and during word testing versus restudy (Van den Broek, Takashima, Segers, Fernández, & Verhoeven, 2013). Our exploratory analysis of source space activity indicates that these memory processes might be linked to theta-band activity in the respective areas. However, to corroborate these findings and to determine the exact source of the obtained activity pattern, future studies should employ other brain imaging techniques (e.g. combined EEG/fMRI recordings), given

the potential role of the hippocampus concerning memory consolidation during sleep, as well as its relationship to theta activity and memory retrieval.

Author note

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Supplementary Information

For

Cueing vocabulary during sleep increases theta activity during later recognition testing

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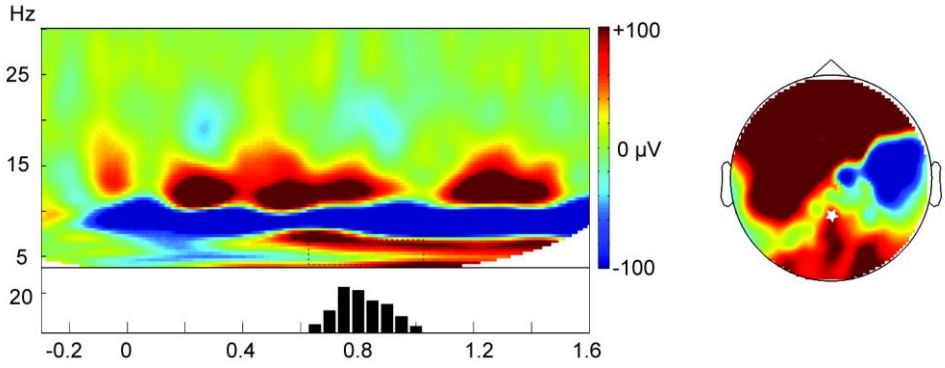
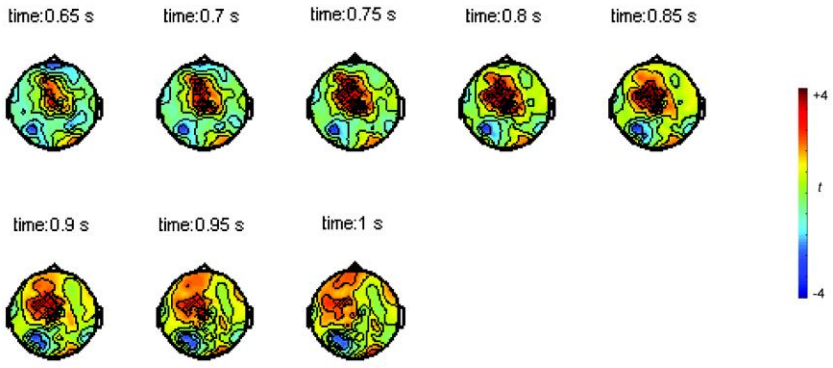
Supplementary Figure 1



Old words, which were successfully encoded before sleep, exhibited significantly stronger theta activity during recognition testing as compared to old words, which were not remembered before sleep. The effect was most pronounced over fronto-parietal areas (here depicted electrode Pz). The bar chart indicates the number of significant electrodes over time

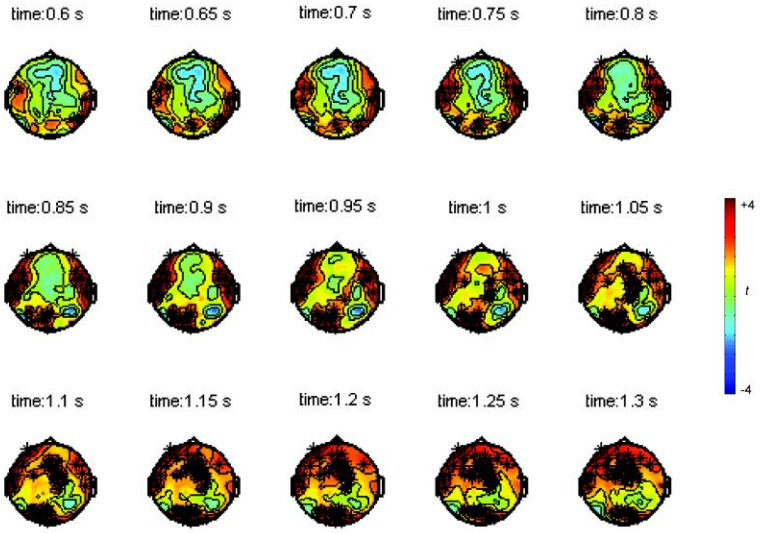
Supplementary Figure 2



Cluster maps indicating significant electrodes for theta power in 50ms steps when comparing old words, which were successfully encoded before sleep and old words, which were not remembered before sleep. Signal increases (warm colors) and decreases (cold colors) indicate *t* values

Supplementary Figure 3

a



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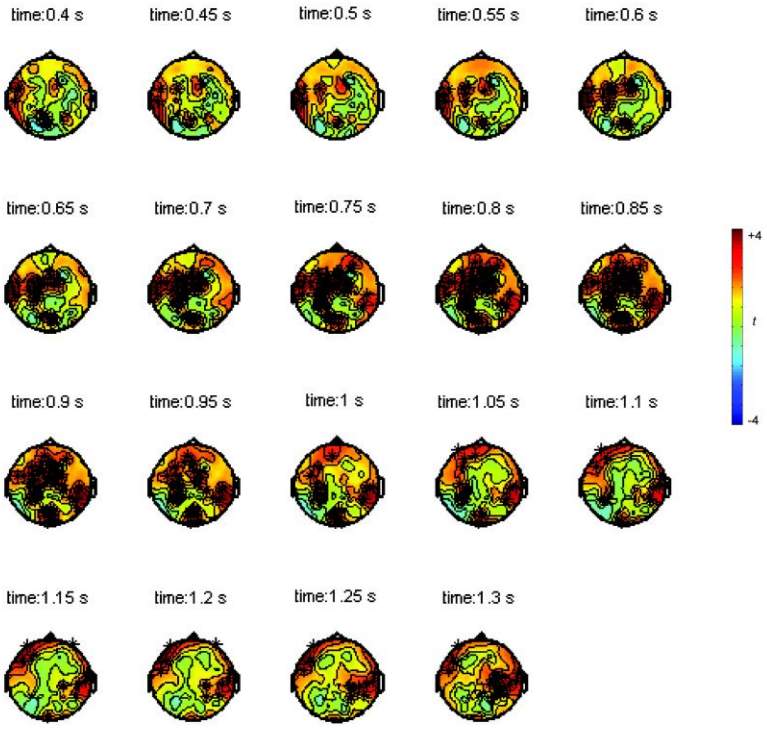
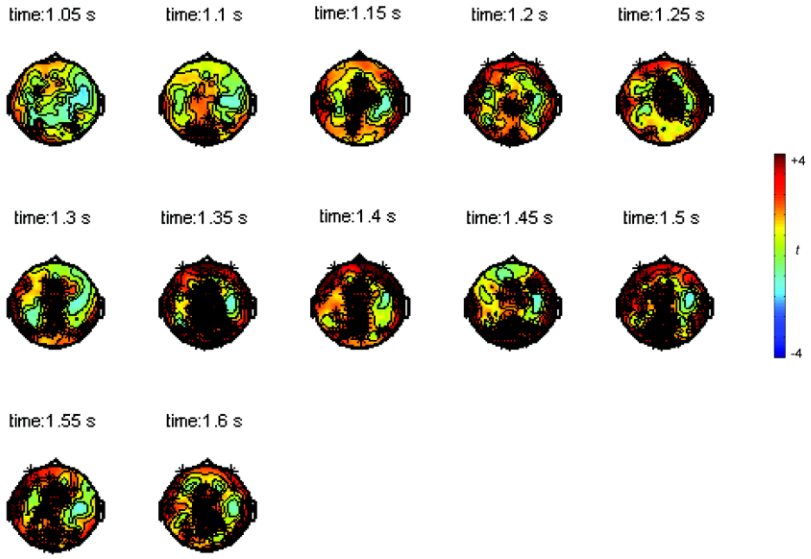


Cluster maps indicating significant electrodes for theta power in 50ms steps, when comparing correctly recognized old words and correct rejections in the reactivation group 1 **(a)** and control group **(b)**.

Signal increases (warm colors) and decreases (cold colors) indicate *t* values

Supplementary Figure 4

a



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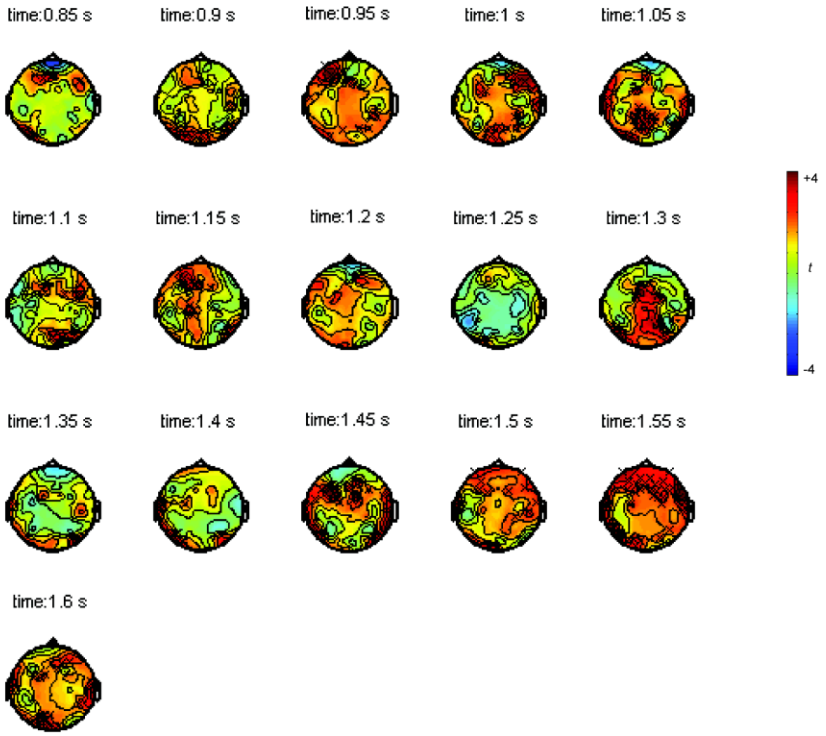

Cluster maps indicating significant electrodes for gamma power in 50ms steps, when comparing correctly recognized old words and correct rejections in the reactivation group **(a)** and control group **(b)**. Signal increases (warm colors) and decreases (cold colors) indicate *t* values

3.2 Manuscript II

Clicking the brain into deep sleep. Commentary on Weigenand et al. (2016)

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Candidate contribution:

Maurice Göldi wrote the manuscript together with Thomas Schreiner.

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Slow-wave sleep (SWS) has been ascribed to play a key role in memory consolidation processes (Rasch & Born, 2013). One of the most salient features of SWS is the elevated occurrence of slow oscillations (< 1 Hz). Slow oscillations (SOs) are generated in thalamic and neocortical circuits and comprise alterations between periods of increased excitability (up-states) and hyperpolarized neural silence (down-states) (Contreras & Steriade, 1995). This alternating neural activity spreads throughout the neocortex and also affects other brain regions amongst others the hippocampus (Contreras, Destexhe, & Steriade, 1997). By orchestrating thalamic sleep spindles and hippocampal sharp-wave ripples (SWR), SOs are thought to co-ordinate the reactivation of memories during sleep, enabling the beneficial effects of sleep on memory formation (Diekelmann & Born, 2010).

Given the assumed role of SOs in mediating the most prominent effects of sleep on cognitive functioning, several attempts have been made to potentiate them (Bellesi et al., 2014).

Recently, Ngo and colleagues (2013) used brief click sounds to enhance SO activity. Critically, the phase of the ongoing oscillatory activity was taken into account to provide auditory stimulation in synchrony with spontaneous SOs. By analyzing the sleep EEG online, the researchers were able to forecast the occurrence of SOs up-states and delivered click sounds time-locked to their appearance. This procedure led to a 9% enhancement of SO power compared to a sham condition, facilitated the induction of SO trains and boosted fast sleep spindle power. Strikingly, memory consolidation processes also benefitted from this procedure. Enhancing SOs led to improved memory for word pairs, which were initially learned before sleep. While those results fit perfectly with the proposed involvement of SOs and sleep spindles in memory consolidation, it is still unclear which specific aspects of the “closed loop” rationale drive the memory enhancement. The work by Weigenand and colleagues (2016) presented in this issue of EJN is one of the first important steps to give these details a closer look. The authors tested whether a similar effect on oscillatory activity and behavioral outcomes would be achieved when applying click sounds in a quasi-phase-dependent manner, a paradigm named “open loop” stimulation. Here, each first click was presented at a random phase of the endogenous present SOs, while the second and third click of a sequence were delivered with a fixed time-interval (~1000ms). The rationale was, that the first click would induce a K-complex (KC), thus a single SO (Steriade & Amzica, 1998), while clicks 2 and 3 would be again presented during the up-states of the evoked SOs in order to enhance SO activity and, presumably, consolidation processes. Thus, the main difference between open and closed loop stimulation concerns the delivery of first clicks. In the closed loop paradigm, first clicks were always preceded by an endogenous

SO and always occurred during a putative SO up-state, while this was not the case in the open loop paradigm.

Presenting click sequences during SWS in an open loop manner enhanced SO power and induced SO trains in a comparable fashion as described in the closed loop account. Furthermore, first clicks also triggered increases in sleep spindle activity, while responses to the second and third clicks were distinctively lower. Still, in harsh contrast to the precursor study, power in slow and fast spindle bands declined during the stimulation period when using open loop stimulation. The decrease in sleep spindle activity was specifically confined to time windows between click intervals (i.e. inter-sequence intervals of 5-9 seconds), while power levels with regards to within-sequence intervals (i.e. during which clicks were delivered) were similar to the sham condition. After all, the key question is whether open loop stimulation would lead to the same memory benefits as it was reported for the closed loop paradigm. Interestingly, no signs of memory enhancement were detectable, as compared to a sham condition.

Thus, although the open loop stimulation successfully induced and enhanced SOs, the anticipated beneficial effect on memory formation was absent. It seems worthwhile to examine at least some of the potential reasons for this functional absence. A crucial question in this context is how closed loop stimulations might accomplish their beneficial effect on memory performance. Endogenous SOs have been repeatedly demonstrated to coordinate SW-Rs in the hippocampus and associated hippocampal reactivation processes (e.g.Peyrache *et al.*, 2011). Furthermore, SOs facilitate the emergence of sleep spindles, which are thought to be involved in the redistribution of hippocampal memories to neocortical sites (Born & Wilhelm, 2012) or the stabilization and strengthening of memory traces after their replay during sleep (Schreiner & Rasch, 2016).

Thus, one might speculate that presenting click sounds in phase with the endogenous SOs, as accomplished in the closed loop paradigm, not only enhances synchronous neural firing of cortical and thalamic neurons, but as well sharpens the interplay of these oscillatory events underlying consolidation processes, thereby unfolding its beneficial effect on memory processing. In contrast, inducing a K-complex out of phase with the spontaneous brain activity, as accomplished with open loop stimulation, might have repeatedly interfered or reset these processes and thereby at least prevented any memory enhancing consequences.

Apart from that, the most visible discrepancy concerning the effects of open and closed loop stimulation on neural oscillations relates to its impact on sleep spindle activity. First clicks in both paradigms induced

strong responses not only in the SO but as well the sleep spindle range. Nevertheless, open loop stimulation led to decreases in sleep spindle activity during the whole stimulation period, which was not the case for closed loop stimulations. This points out once more that the applied procedure interfered with spontaneous brain activity, here specifically the endogenous generation of spindles. Given the fact, that sleep spindles are highly involved in consolidation processes and assumed to protect local reprocessing (Genzel et al., 2014) one might be surprised that such a disturbance did not lead to a severe deterioration in memory performance.

In sum, the fine-grained interplay of diverse oscillatory events during SWS seems crucial when it comes to consolidation processes, while the exact details of their interaction and the functional significance of distinct oscillations still wait for clarification. The random induction of one of the cardinal oscillations acting during sleep (SOs, sleep spindles, SWRs) might therefor underestimate the complexity of the involved processes and in the worst case disrupt them. Thus, in order to enhance not only deep sleep, but as well associated memory processing, it seems inevitable to take the brains spontaneous rhythm into account.

Acknowledgements

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3.3 Manuscript III

Cueing memory during sleep is optimal during slow-oscillatory up-states

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Candidate contribution:

Maurice Göldi was involved with planning the experiment and implemented the technical setup. He also analyzed the data and wrote the manuscript. See section 'Author Contributions' for details.

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Abstract

Slow oscillations play a major role in neural plasticity. It is assumed that slow oscillatory up-states represent crucial time windows for memory reactivation and consolidation during sleep. Here we experimentally tested this assumption by utilizing closed-loop targeted memory reactivation (closed-loop TMR): Healthy participants were re-exposed to prior learned foreign vocabulary during up- and down-states of slow oscillations, respectively, in a within-subject design. We show that presenting memory cues during slow oscillatory up-states robustly improves recall performance, whereas memory cueing during down-states did not result in a clear behavioral benefit. On a neural basis successful memory reactivation during up-states was associated with a characteristic power increase in the theta and sleep spindle band. Such increases were completely absent for down-state memory cues. Our findings provide experimental support for the assumption that slow oscillatory up-states represent privileged time windows for memory reactivation, while the interplay of slow oscillations, theta and sleep spindle activity promote successful memory consolidation during sleep.

Keywords: targeted memory reactivation, closed-loop stimulation, memory consolidation, slow oscillation, theta, sleep spindles, sleep

The consolidation of memories critically depends on hierarchically nested oscillatory brain mechanisms. It has been proposed that the systematic interaction of neocortical slow oscillations (SOs), thalamic sleep spindles and hippocampal sharp wave ripples (SWRs) during sleep reflect the mechanistic vehicle of memory reactivation and thus consolidation during sleep (Rasch & Born, 2013). The <1 Hz SO represents the most prominent signature of slow wave sleep (SWS) and is generated in neocortical and thalamic circuits. SOs comprise alternations between periods of neuronal membrane hyperpolarization, accompanied by widespread neuronal silence ("down-states"), followed by depolarized neuronal "up-states", accompanied by sustained firing (Steriade, Nuñez, & Amzica, 1993). Critically, SOs are thought to coordinate spontaneous memory reactivation processes during sleep, by providing the temporal frame for active memory consolidation (Diekelmann & Born, 2010). It is assumed that the SO up-states drive memory reactivation in the hippocampus together with sharp wave ripples and thalamo-cortical sleep spindles (Sirota & Buzsáki, 2005). The formation of spindle-ripple events is suggested to enable the hippocampal-neocortical dialog and the redistribution of reactivated hippocampal memory information to neocortical long-term stores (Lörincz & Buzsáki, 2000; Staresina et al., 2015).

Despite these theoretical predictions, the mechanistic role of SO up-states for memory reactivation during sleep in humans remains ambiguous. In general, the functional significance of sleep-related memory reactivation in humans has been demonstrated by a series of studies showing that inducing reactivation processes experimentally (targeted memory reactivation; TMR) improves the consolidation process and thereby affects subsequent recall performance (Oudiette & Paller, 2013). TMR studies follow the rationale that memory cues associated with prior learning are presented again during subsequent non rapid eye movement (NREM) sleep to trigger reactivation processes and consequently boost later memory performance. This approach has repeatedly proven successful for context cues such as odors (Rasch, Büchel, Gais, & Born, 2007; Rihm, Diekelmann, Born, & Rasch, 2014; Suss, Gaylord, & Fagen, 2012) and for specific item cues such as sounds (Cairney, Lindsay, Sobczak, Paller, & Gaskell, 2016; Groch, Schreiner, Rasch, Huber, & Wilhelm, 2017; Rudoy, Voss, Westerberg, & Paller, 2009), melodies (Antony, Gobel, O'Hare, Reber, & Paller, 2012; Cousins, El-Deredy, Parkes, Hennies, & Lewis, 2014; Schönauer, Geisler, & Gais, 2014) or verbal material (Lehmann, Schreiner, Seifritz, & Rasch, 2016; Schreiner, Lehmann, & Rasch, 2015; Schreiner & Rasch, 2015a). Importantly, all of these studies presented the memory cues at random points in time during NREM sleep, without taking the on-going oscillatory activity, specifically the phase of SOs, into account. Given the assumed role of SO up-states in driving memory reactivations during sleep, we hypothesized that experimentally aligning the memory cues to the initiation of SO up-states (negative-to-positive transition of the surface slow-wave; see (Vyazovskiy, Cirelli, & Tononi, 2011; Vyazovskiy et al.,

2009)) should be critical for successful TMR, resulting in improved retrieval performance after sleep. In contrast, presenting memory cues at the onset of the SO down states (positive-to-negative transition in the surface slow-wave) should block the memory benefit of TMR. We therefore used SO phase-specific targeted memory reactivation (closed-loop TMR) to test the functional role of SO up-states for memory consolidation in humans. To investigate how closed-loop TMR influences the reactivation and consolidation of memories, we used a vocabulary learning task (Schreiner et al., 2015; Schreiner & Rasch, 2015a). After learning 120 Dutch-German word pairs, 16 healthy young participants slept for 3 hours in the laboratory (see 'Materials and Methods' section, for details). We applied an online SO detection algorithm to present subsets of the prior learned words either during the presence of SO up-states or down-states. As a control condition some of the prior learned words were not replayed at all (uncued words). After sleep participants were tested on memory for the German translations by a cued recall procedure (see Fig. 2a, for a summary of the procedure). We show that words presented during SO up-states were associated with a robust improvement in recall performance compared to uncued words. On a neural basis, successful up-state TMR was associated with characteristic cue-related increases in theta and sleep spindle power. Words replayed during SO down-states did not show this distinct oscillatory pattern of successful memory reactivation and also no significant performance improvement.

Results

Accuracy of the Closed-Loop Algorithm

We first examined whether our algorithm correctly distinguished between words replayed during up- and down-states. We calculated ERPs separately for cues presented in the down-to-up phase transition of the cortical slow wave (targeted area for the up-state cues) and for cues presented in the up-to-down-phase transition (targeted area for the down-state cues, Figure 1a). As expected, the ERP analysis confirmed that up and down state cues were played at highly distinct times of the cortical slow wave and targeted the expected areas (Figure 1b; see Supplementary Figure 2 for ERPs differentiated by remembered and non-remembered words). Despite the pre-stimulus peaks having clearly opposite polarities, post-stimulus ERPs of the two cueing conditions followed a similar temporal evolution (see supplementary section 'Algorithm Accuracy' for further details). To further assess the accuracy of the SO detection algorithm, we determined the phase of the cortical SO at stimulus onset. On a subject level, up-state cues were associated with a mean phase angle of 338.60° ± 20.46°, while down-state cues had a mean phase angle of

132.31° ± 18.46° (see Figure 1c, top row and Figure 1c, bottom row, for results at the trial-by-trial level). Thus, the onset of our memory cues corresponded very accurately with the early phases of the targeted areas, assuring that mostly the whole word length (~400 ms) was played during the intended SO state (see Figure 1a for an overview and Supplementary Figure 4 for a phase analysis of each individual subject).

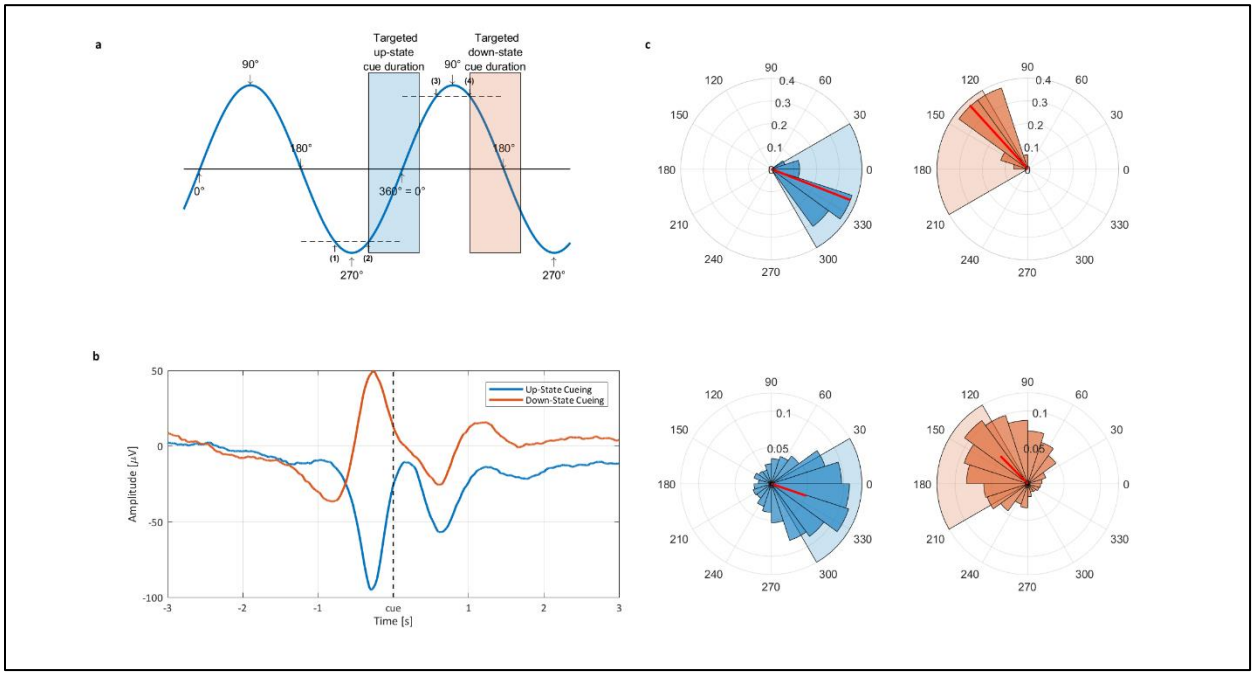

Figure 1| Closed-loop TMR algorithm evaluation. a) Schematic overview of the slow-wave detection algorithm and the targeted areas for up-state TMR (blue) and down-states TMR (red). b) The ERP-analyses revealed that up-state cues were located at the down-to-up transition of the cortical slow wave (beginning of slow oscillatory up-state), and that down-state cues were played at the up-to-down transition (beginning of slow oscillatory down-state). c) Phase angle at stimulus release. The top row illustrates the angles averaged at subject level, the bottom row shows results on trial level. The left column indicates the up-state phases, while the right column shows the down-state phase angles. All data is shown for electrode Fz.

Behavioral Results

As predicted, we observed a robust improvement of memory performance when word-cues were presented during the up-state of the slow oscillation (see Figure 2b): After the sleep interval participants remembered 99.3 ± 2.89% of the prior learned words which were presented during SO up-states, whereas they remembered only 90.92 ± 3.14% of the uncued words (t_{15} = 2.62; *P* = 0.019, two-tailed). In contrast, memory for words presented during SO down-states (96.83 ± 4.27%) did not differ from uncued words (t_{15} = 0.93, *P* = 0.366). On a descriptive level, memory performance for words presented during down-states was just in-between up-state and uncued words. Furthermore no difference to memory performance associated with up-state cued words was observable (t_{15} = 0.43, *P* = 0.673). It must be noted that

the variance was also descriptively higher for words cued during the down-state than the other two categories (down: 292.29; up: 133.20; non-react: 157.58). In addition, down-state cued words were negatively correlated with memory for uncued words (r(14) = -0.45, *P* = 0.079). This coefficient differed significantly (Z = 2.34, *P* = 0.015) from the positive correlation between up-state cued words and uncued words (r(14) = 0.44, *P* = 0.091). Consequently, the overall multivariate analysis of variance including all three word categories simultaneously only reached a statistical trend ($F_{(2,14)}$ = 3.21, *P* = 0.071).

Furthermore, we explored the associations between memory performance and time spent in the different sleep stages (for descriptive values of sleep stages, see Table 1). Interestingly, only participants with high amounts of rapid eye movement (REM) sleep profited from down-state cueing, while participants with low or no REM sleep did not (r(14) = 0.59, *P*= 0.017; see Figure 2c; please note that no cue was presented during REM sleep). We observed no other significant correlations between any sleep stage and memory performance in any word category (all *P* > 0.05). We also observed no significant correlation between the number of cues played during NREM sleep and memory performance, neither in the up- nor down-state category (both, *P* > 0.180). Descriptively, each person received 308.94 ± 18.98 cues during the night, which corresponds to about 5 repetitions of all cued words, with 50.29 ± 0.21% up-state cues and 49.71 ± 0.21% of the words presented during down states.

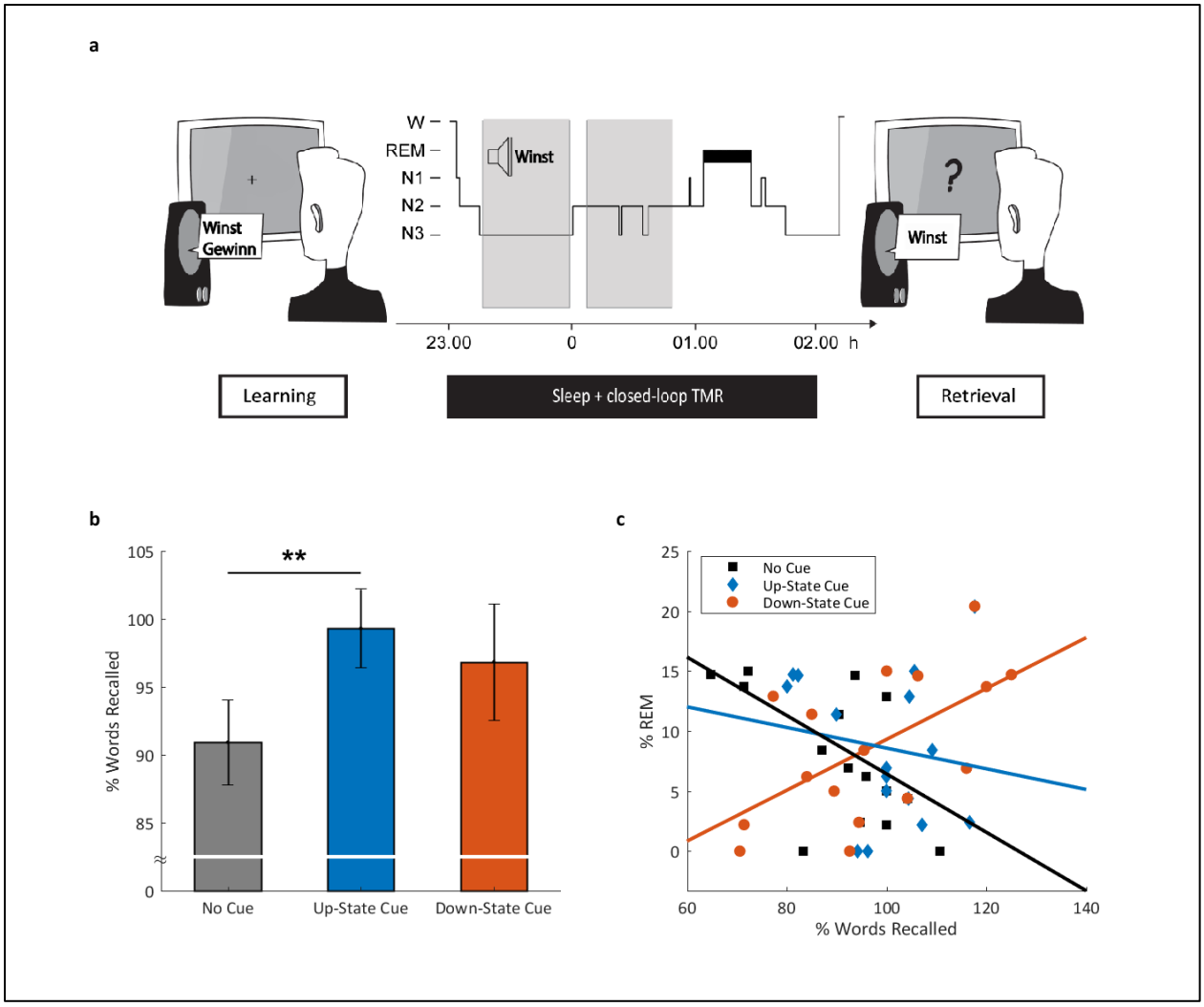

Figure 2| Experimental Procedure and memory task results. a) After studying 120 Dutch-German word pairs in the evening, participants slept for 3 hours. During NREM sleep, 40 Dutch words were presented during SO up-states and 40 Dutch words were presented during SO down-states using closed-loop TMR. 40 Dutch words were not replayed. A cued recall procedure was applied after sleep, testing the participant's memory for the German translations. b) Presenting prior learned words during SO up-states significantly enhanced memory performance compared to uncued words. Recall performance of words replayed during SO down-states did not differ from the two other categories. Retrieval performance is indicated as percentage of recalled German translations with performance before sleep set to 100%. Values are mean ± s.e.m. **P ≤ 0.025. c) Correlation between memory performance and relative time spent in REM sleep. Memory performance for words presented during down-states is positively correlated with time spent in REM sleep ($r(14) = 0.59$, $P = 0.017$). There was no significant correlation for words presented during up-states ($r(14) = -0.16$, $P = 0.552$), and a marginal significant negative correlation for uncued words ($r(14) = -0.49$, $P = 0.052$).

Total sleep duration [min]	% WASO	% N1	% N2	% SWS	% REM
181.84 ± 8.55	5.54 ± 2.76	4.75 ± 0.83	48.41 ± 2.71	31.99 ± 2.54	8.64 ± 1.54

Table 1| Sleep parameter. N1, N2: NREM sleep stages N1 & N2, SWS: slow-wave sleep (N3), REM: rapid eye movement sleep, WASO: wake after sleep onset. Values are means ± s.e.m.

Oscillatory Results

Based on our previous reports (Schreiner et al., 2015; Schreiner & Rasch, 2015a), we focused the time-frequency analysis on oscillatory power in the theta band (5-8 Hz) and the sleep spindle band (11-15 Hz) between the time points 0 ms and 2000 ms after stimulus onset. For memory cues played during the up-state we observed a significant increase in theta power for later remembered compared to later non-remembered words between 920 and 1480 ms involving a cluster of 22 channels ($P = 0.05$, see Figure 3a). The significant electrodes had a right central distribution (see Figure 3b left column, bottom row). Also in the spindle band, the overall analysis revealed a significant increase in spindle power for remembered compared to non-remembered words between 830 and 1770 ms involving all 31 electrodes ($P = 0.023$, see Figure 3b left column, top row). In contrast to cues presented during SO up-states, we did not observe any significant power differences for remembered vs. non-remembered words played in the SO down-state, neither for the theta ($P > 0.30$) nor the spindle band ($P > 0.60$, see Figure 3c and d). Even a more restricted test-statistics limited to the time-range of the up-state clusters revealed no significant effect (not shown; theta: $P = 0.213$, spindle: no cluster found). The general oscillatory differences between up- and down-state cueing are shown in Supplementary Figure 5 and discussed in the supplementary results section ‘Oscillatory Analysis Up versus Down’.

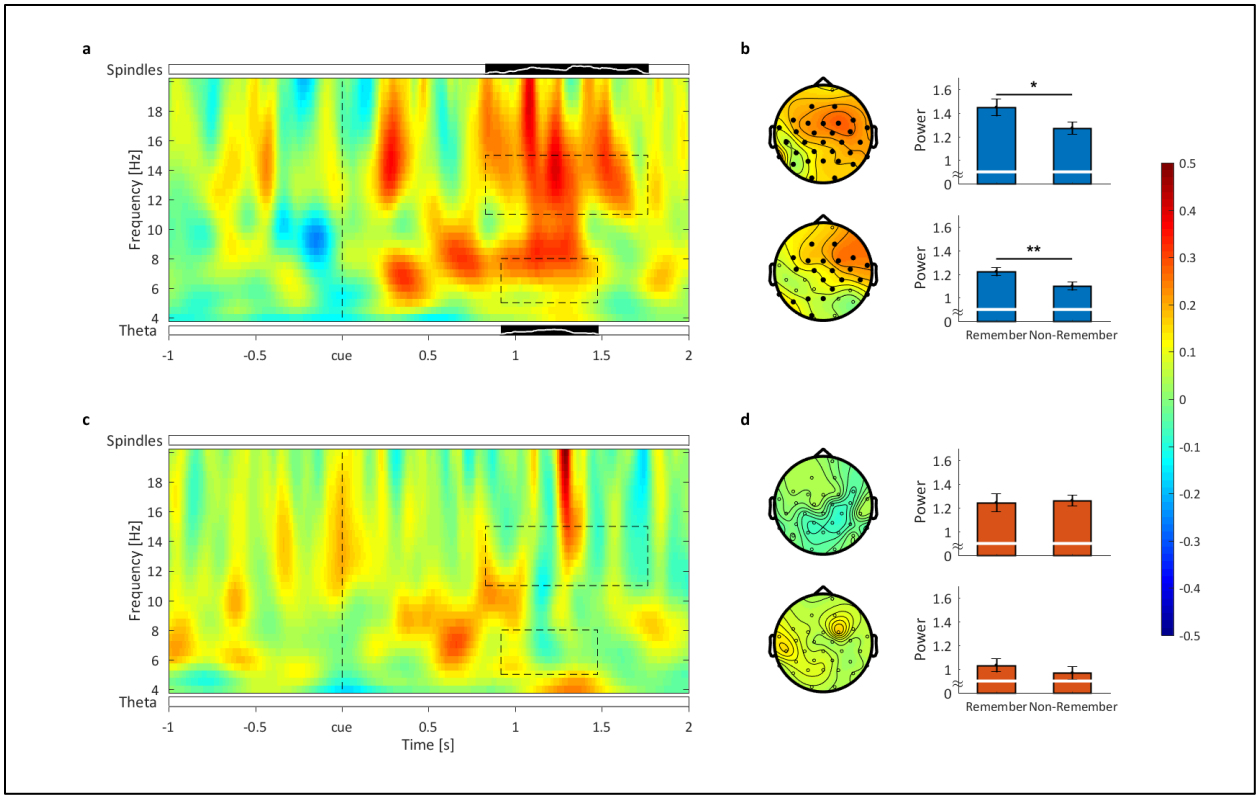

Figure 3| Oscillatory results. Time-frequency contrasts between remembered and not-remembered words in the theta and sleep spindle band for a) up-state and c) down-state cues, shown for the representative Fz electrode. Black bars (significant cluster in frequency band analysis) with white lines below and above the time-frequency plot show the number of significantly differing electrodes for the theta and sleep spindle band respectively. The full height of the bar corresponds to 100% (31) electrodes. Dashed boxes indicate the areas of significant difference between remembered and not remembered words. These time-windows were used to illustrate the topographical distributions (b and d) left column, top row spindle band, bottom row theta band; significant electrodes shown as filled black dots). b) and d) right column show the mean power within the significant clusters, averaged over the significant electrodes, all frequencies and time in the sleep spindle (top) and theta (bottom) band. For up-state cueing a) remembered words show enhanced power in the theta (5-8 Hz) as well as the sleep spindle (11-15 Hz) range compared to not-remembered words. Averaged over time duration, channels and frequency band, within these clusters this difference was highly significant in the theta band (t_{15} = 2.63, P = 0.01; see b right column, bottom row) and in the spindle band (t_{15} = 2.37, P = 0.016; see b, right column, top row). For words presented during down-states c) no significant difference emerged between remembered and forgotten words, neither in the sleep spindle nor the theta band. Consequently, averaged activity in those clusters observed in the analysis of SO up-states did not reveal any significant differences for down-state cues, neither in the theta (t_{15} = 1.02, P = 0.161) nor the spindle band (t_{15} = -0.33, P =0.627). Mean ± SEM are indicated. **: P < 0.01; *: P < 0.05.

Discussion

The present study investigated for the first time the impact of SO phase-dependent memory reactivation during sleep on memory consolidation. We used a simple, yet effective algorithm to target the presentation of prior learned words specifically into the transition between neuronal quiescence and synchronized neuronal activity and vice versa (i.e. SO up and down-states). We demonstrate that presenting memory

cues during the presence of SO up-states significantly improved memory performance compared to uncued words. In contrast, cueing words during SO down-states did not exhibit such a clear beneficial effect. However, participants who spent more time spent in REM sleep after NREM cueing showed a stronger memory benefit from cues presented during down-states. Our oscillatory analyses demonstrate that successful TMR during up-states was related with higher theta and spindle band activity than non-successful TMR as described previously (Groch et al., 2016, 2017; Lehmann et al., 2016; Schreiner et al., 2015; Schreiner & Rasch, 2015a). This characteristic oscillatory pattern of successful memory reactivation during NREM sleep was not observable for the down-state condition.

In our study, we targeted the SO transition from down- to up-state (down state condition) and up- to down-state (up-state condition) to specifically reactivate memories during sleep. We aimed for these transition periods as it has been shown that neuronal down-states occur slightly before the negative peak of the surface slow wave, while neuronal up-states start with the negative-to-positive transition of the cortical slow-wave (Vyazovskiy et al., 2011, 2009). Our algorithm accurately presented the memory cues in the intended target areas. In addition, our ERP analysis shows that, while the signals of the two cue targets differed strongly before cue onset, they followed a similar temporal evolution after cue presentation, indicating that auditory cues pushed the brains neuronal population into similar temporal successions as measured by surface electroencephalogram (EEG), regardless of the underlying endogenous brain state.

Taken together, our results suggest that SO up-states represent an optimal time window for targeted memory reactivation and accompanying consolidation. In contrast, TMR during down-states did not ultimately block but mostly attenuated the chances of successful memory consolidation.

Several theoretical considerations (Mölle, Bergmann, Marshall, & Born, 2011; Mölle, Marshall, Gais, & Born, 2002; Mölle & Born, 2011; Sirota & Buzsáki, 2005; Staresina et al., 2015; Steriade, 2006) lead to the prediction that SO up-states drive the reactivation of memories during sleep, thereby representing a critical time window for memory consolidation. During slow oscillatory up-states, more and more neurons fire synchronously (Vyazovskiy et al., 2009). This synchronous firing leads to a higher probability of activating a cascade of downstream–connected neurons. Memory traces are thought to be stored in the brain as interconnected neurons (Liu et al., 2012; Reijmers, Perkins, Matsuo, & Mayford, 2007; Silva, Zhou, Rogerson, Shobe, & Balaji, 2009). By randomly activating a large portion of neurons during the up-state, these interconnected neurons (i.e. memory traces) fire together with a high probability (Diekelmann & Born, 2010). We suggest that presenting auditory cues during the presence of SO up-states, activates

neurons of the associated memory trace with a heightened probability. In combination with the heightened random neural activations of the up-state, a neuronal cascade involving the complete memory trace is triggered with increased probability. Interestingly, ERP associated with up-state TMR indicated a regular, uninterrupted 1Hz oscillation after cue onset, possibly supporting the endogenous reactivation and consolidation mechanism , while down-state TMR seemed to disrupt the ongoing SO pattern and lead to a delayed negative trough of the SO wave.

Still, presenting memory cues during the presence of a down-state might also activate the corresponding memory trace with an above chance probability. However, as the brain is at this point in a quiescent state, the reactivation of the specific memory trace might not be fully supported by the endogenous activity of the brain. The chance of reactivating a memory trace during the down-state, therefore remains inferior to up-state reactivation. Also, the natural continuation of the 1 Hz oscillation associated with down-state cueing seemed to be disturbed through stimulus presentation, leading to a slight delay in the subsequent negative ERP deflection relative to its undisturbed form. Nevertheless, both cueing time points seem to increase the chance of reactivating a memory relative to random endogenous chance reactivations.

In contrast to our experimental findings, a recent analysis by Batterink and colleagues (2016) identified the SO down-state to represent the optimal phase for TMR. In a post-hoc evaluation of two previous TMR studies (Creery, Oudiette, Antony, & Paller, 2015; Rudoy et al., 2009), the authors found that the amount of forgetting was lowest for items presented just before the onset of down-states. The authors explained this rather surprising result (i.e. that the optimal phase for TMR was found to occur quite far in advance to the anticipated one, the SO up-state), by a potential time lag caused by auditory stimulus processing and concluded that a closed-loop TMR approach would shed further light on these findings. Our results indicate that the optimal phase for TMR and thereby memory reactivation is during down- to up-transition of the SO. However, general differences between the studies (e.g. utilized task, differing sound cues etc.) could potentially also account for the timing discrepancies. Our study was specifically designed to test the difference between up-state, down-state and non-cued words on memory performance. Thus, our algorithm targeted the early stage of the up- to down- and down- to up-transition and did not aim for the pre-down-state peak interval, which was found preferable by Batterink and colleagues (2016).

An interesting finding of our current work is that cueing success for down-state TMR was positively correlated with the relative time spent in REM sleep. Two recent studies have shown, utilizing an afternoon nap design, that REM sleep systematically influenced the effect of TMR on new word learning (Laura J. Batterink & Paller, 2017) and the integration of newly learned spoken words (Tamminen, Lambon Ralph,

& Lewis, 2017). This pattern of result led the authors to assume that TMR during NREM sleep might reactivate a certain memory trace, and at the same time prepare it for integration into pre-existing associative networks during the next REM sleep cycle (which might be associated with de-stabilization of a given memory trace during NREM sleep, but see (Diekelmann, Büchel, Born, & Rasch, 2011)). In the light of these results we propose that memory reactivations targeted into the optimal SO up-state successfully reactivate and stabilize the memory trace immediately through the critical interplay of SO, theta and sleep spindle activity. In contrast, memory cues targeted into suboptimal (non-up-) states also have the chance to reactivate the memory trace but lack crucial stabilization processes, possibly due to the marginal spindle activity. These memory traces are therefore dependent on the re-stabilization during REM sleep. Furthermore, random TMR during an afternoon might be specifically vulnerable to presenting cues during suboptimal states due to shallower afternoon sleep than nighttime sleep.

While the role of REM sleep in stabilizing memory representations was specifically associated with down-state TMR, successful up-state TMR was directly linked with oscillatory power increases in the theta and sleep spindle range. A growing number of TMR studies (Farthouat, Gilson, & Peigneux, 2017; Groch et al., 2016; Lehmann et al., 2016; Oyarzún, Morís, Luque, de Diego-Balaguer, & Fuentemilla, 2017; Schreiner et al., 2015; Schreiner & Rasch, 2015b) report such elevated levels of oscillatory theta and sleep spindle power to be tightly associated with cueing success, indicating a critical role of both frequency bands for the reactivation and stabilization of memories during sleep. According to our working model, increases in theta power indicate successful reactivation of the memory by cueing, whereas related increases in spindle power support the consolidation and integration of reactivated memory into cortical networks for long-term storage(Schreiner & Rasch, 2017). Here we observed significant increases in theta power for remembered as compared to non-remembered words from 0.92 to 1.48 seconds with regard to up-state cues, thus exhibiting some overlap concerning the first positive ERP-peak after stimulus onset. This temporal pattern also lies within the potentially crucial 1.5 second post-stimulus time window identified in our previous work (Schreiner et al., 2015), in which consolidation processes are prone to interference, leading to a blockade of associated reinstatement processes. There was no difference between remembered words and non-remembered words in theta activity with regard to down-state cues.

In addition, we found increase in sleep spindle power between 0.83 and 1.77 seconds after up-state cues, similarly coinciding with the first positive ERP-peak after stimulus onset, while no difference in spindle power was observable in the case of the down-state cueing. Thus, up-state cues, which were played in accordance with the endogenous rhythm of the brain, seem to recruit sleep spindles more easily than

down-state cues, thereby enhancing memory consolidation in a more robust fashion. Sleep spindles are assumed to promote the re-distribution of reactivated memory representations to neocortical sites (Born & Wilhelm, 2012), with hippocampal reactivation signals being nested in individual spindle troughs (Mölle, Eschenko, Gais, Sara, & Born, 2009; Siapas & Wilson, 1998; Sirota & Buzsáki, 2005; Staresina et al., 2015). Furthermore, inducing thalamic sleep spindles, when phase-locked to SO up-states enhances the oscillatory coupling between SOs, spindles and hippocampal ripples and furthermore memory consolidation (Latchoumane, Ngo, Born, & Shin, 2017). However, memory reactivation processes in rodents seem to slightly precede the appearance of sleep spindles (Peyrache, Khamassi, Benchenane, Wiener, & Battaglia, 2009), while cortical sites are presumably even shut off from hippocampal inputs during the presence of sleep spindles (Peyrache, Battaglia, & Destexhe, 2011). This might suggest that sleep spindles themselves rather enable locally undisturbed cortical reprocessing of reactivated memories (Genzel, Kroes, Dresler, & Battaglia, 2014). Still, there is little doubt that sleep spindles are tightly linked to slow oscillatory activity and consequently memory processing during sleep.

Bolstering this assumption, Ngo and colleagues (H.-V. V. Ngo et al., 2015; Hong-Viet V Ngo, Martinetz, Born, & Mölle, 2013) have previously demonstrated that entraining SOs through closed loop auditory stimulation, enhances phase-locked spindle activity and importantly memory recall after sleep. Interestingly, the associated increases in sleep spindle amplitude were positively correlated with later memory performance. These studies were able to elegantly demonstrate that elevating activity in the SO and phase-locked sleep spindle range by stimulation of SO up-states leads to a general improvement in memory performance. However, whether these effects resulted from specifically enhancing reactivation processes through up-state stimulation remained unknown. In our study, we are able to test this relation more directly by targeting the memory content specifically into the proposed functional up-state of the SO and compare this to TMR in the SO down-state. As pointed out above we also found an increase in spindle power for up-state cues when contrasting later remembered and non-remembered words, providing a more direct link between SO phases, spindles and memory performance. The SO phase-specificity of sleep spindles has been shown consistently in the existing literature (e.g. (Klinzing et al., 2016; Staresina et al., 2015)), as well as their conduciveness for the stabilization of memories (Clemens, Fabó, & Halász, 2005; Schabus et al., 2004). In a recent study, Weigenand and colleagues (2016) induced slow oscillations by presenting repeated tones in an open-loop stimulation paradigm. Here, an initial tone was played into a random SO phase, evoking a SO or K-complex with high probability and re-setting the SO to a known phase. Subsequent tones where then played into SO up-states. Interestingly, this approach led to de-

creases in spindle power and no beneficial effect on memory performance compared to a control condition. Similarly, in our experiment, when presenting word-cues during SO down-states we forced the signal into a slow oscillatory rhythm, while neither a memory specific spindle response nor a stable memory enhancing effect became apparent. This might further indicate that inducing a SO out of phase has a reduced probability of triggering memory consolidation relevant processes.

In sum, our results suggest that slow-oscillatory up-states present an optimal time window for benefitting memory by TMR. Still, it must be noted that the impact of up-state associated TMR did not exceed the usually described ~10 percent benefit of memory cueing in previous 'random-phase' TMR studies (Schreiner et al., 2015; Schreiner & Rasch, 2015a). The equivalence concerning the obtained effects might result from the fact that those earlier studies featured a high stimulus repetition rate (~10 repetitions per memory cue compared to ca. 5 repetitions in the current study). In addition, presenting memory cues at suboptimal phase appears to slightly support the consolidation of memories according to our findings, especially when followed by subsequent REM sleep. As random-phase TMR allows for higher stimulus repetitions and is possibly easier to implement, it might even be the method of choice for enhancing memories during nighttime sleep in real-life.

Materials & Methods

Subjects

A total of 22 healthy, right-handed subjects (18 female, mean age = 20.85 ± 0.28) with German mother tongue and without Dutch language skills participated in the study. 6 subjects had to be excluded from the study due to technical reasons (n = 5) or because the subjects were too sensitive to the auditory cues and could not sleep (n = 1).

None of the participants was taking any medication at the time of the experiment and none had a history of any neurological or psychiatric disorders. All subjects reported a normal sleep-wake cycle and none had been on a night shift for at least 8 weeks before the experiment. On experimental days, subjects were instructed to get up at 7:00h and were not allowed to consume caffeine or alcohol or to nap during daytime. All participants spent an adaptation night in the sleep laboratory prior to the experiment. The ethics committee of the Canton of Fribourg approved the study, and all subjects gave written informed consent

prior to participating. After completing the whole experiment, participants received 120 Swiss Francs or course credit for participating in the study.

Design and Procedure

Participants entered the laboratory at 21:00h. The session started with the application of the electrodes for standard polysomnography, including electroencephalographic (EEG; 32 channels, Brain Products GmbH), electromyographic (EMG), and electrocardiographic (ECG) recordings.

The encoding phase started at ∼22:00 h with the learning of Dutch-German word pairs (for a detailed description see 'Vocabulary Learning Task' section). After completing the learning task participants went to bed at 23.00 h and were allowed to sleep for 3 h. During the 3-h retention interval, a selection of the prior learned Dutch words was presented again during sleep stages N2 and SWS. At ∼2.00 h, subjects were awakened from sleep stage 1 or 2 and recall of the vocabulary was tested again (see Figure 2a).

Vocabulary-Learning Task

The vocabulary-learning task consisted of 120 Dutch words and their German translations. There were three learning rounds. In each, Dutch words were presented aurally (duration range 300–500 ms) via loudspeakers (70 dB sound pressure level). In the first learning round, each Dutch word was followed by a fixation cross (500 ms) and subsequently by a visual presentation of its German translation (2000 ms). The inter-trial interval between consecutive word pairs was 2000 – 2200 ms. Subjects were instructed to memorize as many word pairs as possible. In a second round, the Dutch words were presented again followed by a question mark (ranging up to 7 seconds in duration). The participants were instructed to vocalize the correct German translation if possible or to say, "next" (German translation: "weiter"). Afterward, the correct German translation was shown again for 2000 ms, irrespective of the correctness of the given answer. In the third round, the cued recall procedure was repeated without any feedback of the correct German translation. Recall performance of the third round (without feedback) was taken as pre-retention learning performance. Here, participants recalled on average 60.5 ± 11.29 words (range 43 to 82 words) of the 120 words correctly, indicating an ideal medium task difficult (50.42% words remembered).

Reactivation of Vocabulary

Of the 120 words learned before sleep, 2/3 of the remembered and 2/3 of the non-remembered words, totaling 80 words, were randomly selected for cueing during sleep. The remaining 40 words were not replayed during sleep (mean remembered uncued: 20.56 ± 0.95). From all words selected for cueing, half

of the remembered and half of the non-remembered words were randomly selected for up-state cueing and the other half for down-state cueing (mean remembered up-state: 19.88 ± 0.90; mean remembered down state: 20.00 ± 0.95 down-state). Cueing of the Dutch words started after the participants entered stable N2 sleep and was paused as soon as arousals were detected. During the 3 hour retention phase words were presented aurally via loudspeakers (55 dB sound pressure level) either during the up-state of a SO (up-state cueing) or during the down state of a SO (down-state cueing) for a total of 90 minutes.

Online Detection Algorithm

The open-source FieldTrip toolbox (Oostenveld, Fries, Maris, & Schoffelen, 2011) was used to accomplish the online detection of slow oscillations and auditory replay. Slow oscillations were detected on the basis of EEG recordings from electrode site Fz, because SOs usually originate in the prefrontal cortex (Nir et al., 2011). Post-hoc analysis of the phase distribution at cue onset shows an even phase distribution for all channels (See Supplementary Figure 3). The signal was referenced to the average potential from linked mastoid electrodes and filtered between 0.2 and 4 Hz. A custom fieldtrip script running under Matlab enabled to respond to the incoming EEG data from electrode Fz in real time. To detect up- and down-states, respectively, each time the EEG signal crossed an adaptive threshold the auditory replay was triggered. The algorithm was implemented as a finite-state machine (see Supplementary Figure 1.): In the first state the algorithm tries to detect a potential slow wave by waiting for the EEG signal to go below -75 µV ((1) in Figure 1a). If the auditory stimulus is to be played in the up-state, the presentation is triggered as soon as the EEG signal goes back above -75 µV (see position (2) in Figure 1a). On the other hand, if the stimulus is to be released during a down-state, the following state will wait for the signal to pass into the positive domain (above 10 µV) ((3) in Figure 1a) and then again go below the release threshold of 10 µV ((4) in Figure 1a). At this point the auditory stimulus is played. As the duration of all words was ~400 ms, they could fit within their respective target state. After triggering the replay, an 8 second recovery period is entered before the algorithm returns to its initial state.

Recall of Vocabulary after the Retention Interval

During the recall phase, the 120 Dutch words were presented again aurally in a randomized order. The participants were asked to vocalize the correct German translation if possible. As index of memory recall of German translations across the retention interval, we calculated the relative difference between the number of correctly recalled words before and after the retention interval, with the pre-retention memory performance set to 100%.

Sleep EEG

Sleep was recorded by standard polysomnography including EEG, electromyographic (EMG) and electrocardiographic (ECG) recordings. EEG was recorded using a 32-channel system (EasyCap, Brain Products GmbH). Impedances were kept below 10 kOhm. Voltage was sampled at 500 Hz and initially referenced to the vertex electrode (Cz). In addition to the online identification of sleep stages, polysomnographic recordings were scored offline by 3 independent raters according to standard criteria (Iber, Ancoli-Israel, Chesson, & Quan, 2007).

Preprocessing

EEG preprocessing was performed using Brain Vision Analyzer software (version 2.1; Brain Products, Gilching, Germany). Data were re-referenced to averaged mastoids and low passed filtered with a cutoff frequency of 30 Hz. The data was segmented into 6 second segments, beginning 3000 ms before stimulus onset. Trials including artifacts (e.g. movement artifacts) were manually removed after visual inspection.

Afterwards, epochs were categorized into up- and down-state stimuli, depending on whether they were presented during up- or down-states, respectively. Furthermore, all stimuli were differentiated on a behavioral level into 'Remember' and 'Non-Remember' words. Remembered words refer to those words that were remembered at recall after sleep, while non- Remember words were not. Additionally 'Up-All' and 'Down-All' will be used to denote all up-state and all down-state cues irrespective of the behavioral outcome. All further analyses were done using Matlab (The Math Works Inc., Natick, MA, USA) and the FieldTrip toolbox (Oostenveld et al., 2011).

Event-Related Potentials

ERPs were analyzed using FieldTrip. Trials were averaged and baseline corrected for each stimulus category within each subject. For baseline correction the segment from -3 to -2 seconds was used, as the prestimulus time window closer to the cue-onset systematically differs between up- and down-state. Subsequently the ERPs were averaged across all subjects for each stimulus category.

Slow Wave Phase Analysis

The preprocessed data was low-pass filtered at 1.5 Hz and a Hilbert transform was applied. The angle information was then averaged within each behavioral category for each subject. Descriptive and inferential statistics were calculated using the Circular Statistics Toolbox (Berens, 2009).

Time-Frequency Analysis

Time-frequency analysis was performed using FieldTrip. To obtain oscillatory power we used a continuous wavelet transformation (complex Morlet waveform, 5 cycles) for frequencies ranging from 0.5 to 20 Hz, in steps of 0.5 Hz and 10 ms. The frequency data was normalized using data from -3 to -2 seconds pre-stimulus as baseline for each stimulus category. Then the trials were averaged for each subject.

Statistical Analysis

We analyzed the behavioral data using MANOVAS. We used post hoc paired t-tests corrected for two-sided testing. Pearson's linear correlation coefficient was computed. A threshold of $P = 0.05$ was used to set statistical significance. For the time-frequency analysis we tested the difference between the remembered and non-remembered words with a cluster based permutation test with dependent samples and a cluster level alpha of 0.05. Monte Carlo p-values were computed on 1000 random data partitions. The critical alpha-level was set to 0.05. We first tested for significant clusters broadly from 0 to 2 seconds after stimulus onset, across all channels and across all frequencies (0.5 to 20 Hz), correcting for two-sided testing. In a next step, we specifically tested the frequency bands of interest (i.e. averaged theta power: 5 to 8 Hz; averaged spindle power: 11 to 15 Hz) for positive clusters, as both frequency bands have been shown to be related to cueing success in previous studies (Farthouat et al., 2017; Groch et al., 2017; Lehmann et al., 2016; Oyarzún et al., 2017; Schreiner et al., 2015; Schreiner & Rasch, 2015a). Testing was done independently for up- as well as down-state trials. We also tested Up-All versus Down-All to obtain the general oscillatory differences between the up-state and down-state TMR.

Author Contributions

T.S., M.G. and B.R. designed the experiment, E.vP. and T.S. carried out the experiments, M.G.,T.S. and E.vP. analyzed the data and M.G., T.S. and B.R. wrote the paper.

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Competing Financial Interests

The Authors declare no competing financial interests.

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Supplementary Information

for

Cueing memory during sleep is optimal during slow-oscillatory up-states

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Supplementary Results:

Algorithm Accuracy

The auditory stimulus cues were targeted into the transition of down- to up-state or up- to dow-state for the up- and down-state cues respectively. Visual inspection of the ERP confirms the precision of the algorithm. The auditory signal had an effect on the further evolution of the ERP signal. For up-state cues, the negative post-stimulus peak highly resembles an endogenous ~1Hz slow wave. In contrast, down-state cues disrupted the natural continuation of the spontaneous slow wave. While the pre-stimulus peaks show no temporal lag (t_{15} = 0.07, *P* = 0.942), the timing of the post-stimulus ERP peaks differ significantly (t_{15} = 3.00, *P* = 0.009 for the negative peak; t_{15} = 2.31, *P* = 0.036 for the positive peak). For both the negative and positive peaks, the down-state peaks are 11 ms earlier than the up-state peaks.

Oscillatory Results Up versus Down

To assess the general oscillatory difference between up and down states, we ran a time frequency analysis for time-points ranging from 1 second pre-stimulus to 2 seconds after stimulus onset and including frequencies from 4 to 20 Hz, contrasting all up-state cues versus all down-state cues (see Supplementary Fig. 5a). Results revealed a significant positive cluster spanning the whole segment in time, frequency and channels (*P* = 0.001). Therefore we tested for frequency band specific positive clusters in the theta (5-8 Hz) and sleep spindle (11 – 15 Hz) band across the same time range and all electrodes (see Supplementary Fig. 5a below and above time-frequency plot). We found a significant cluster from -0.64 seconds to 2 seconds in the theta band (*P* = 0.001). In the spindle band the cluster spanned from -1 to 1.2 seconds (*P* = 0.001). Both clusters involved all channels. To better understand the power relationship between the up- and down-state within the frequency bands of interest, we compared the average power of the up-and down-state for a pre-stimulus and a post-stimulus section in the theta (pre: -640to -120 ms; post: 320 to 670 ms) and spindle band (pre: -1000 to -580 ms; post: 0 to 400 ms). The segments were chosen visually, based on the strength of power difference in the respective frequency bands at electrode Fz (see Supplementary Fig. 5a; segments marked as dashed boxes). The up-state cues show an increased power both pre- and post-stimulus (t_{15} = 8.23, *P* < 0.001 and t_{15} = 7.99, *P* < 0.001 respectively) in the theta as well as the spindle band (t_{15} = 2.67, *P* = 0.009 and t_{15} = 7.57, *P* < 0.001 respectively). Thus, all comparisons revealed a significantly elevated power-level for up-state cues compared to down-state cues (see Supplementary Fig. 5c).

Supplementary Table 1: Word list for the memory task

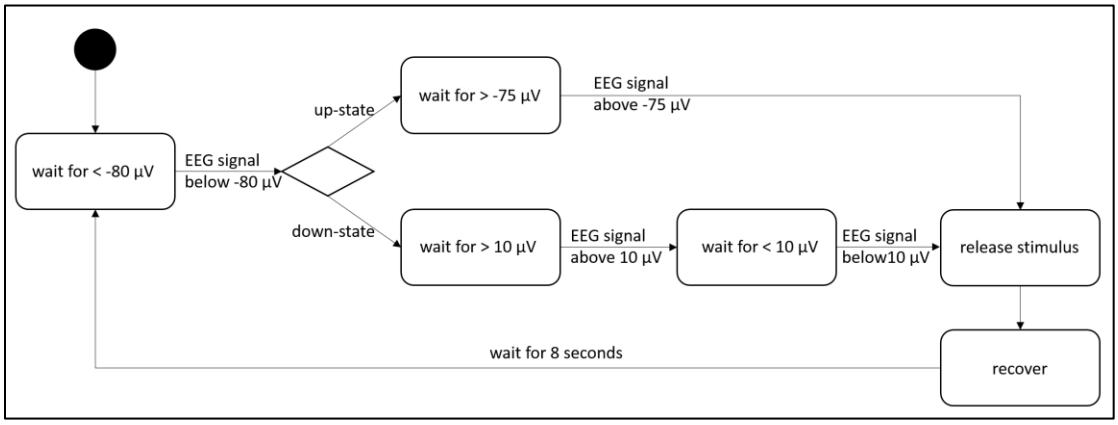
Dutch	German	English	duim	Daumen	thumb
aap	Affe	monkey	eed	Eid	oath
baan	Beruf	job	fles	Flasche	bottle
beek	Bach	brook	fout	Fehler	fault
been	Bein	leg	geur	Geruch	odor
bel	Klingel	bell	gif	Gift	poison
beurs	Börse	stock market	hak	Absatz	heel
bij	Biene	bee	hei	Heide	heath
blik	Blech	sheet metal	hiel	Ferse	heel
bloem	Blume	flower	hout	Holz	wood
bloes	Bluse	blouse	hulp	Hilfe	help
boek	Buch	book	hut	Hütte	hut
boer	Bauer	farmer	inkt	Tinte	ink
bol	Kugel	sphere	jas	Jacke	jacket
boom	Baum	tree	kast	Schrank	closet
bos	Wald	forest	kerk	Kirche	church
bout	Bolzen	bolt	kok	Koch	cook
brug	Brücke	bridge	kras	Kratzer	scratch
buik	Bauch	belly	kruk	Krücke	crutch
buks	Büchse	rifle	kus	Kuss	kiss
dak	Dach	roof	kust	Küste	coast
deel	Teil	part	kwal	Qualle	jellyfish
deur	Tür	door	lens	Linse	lens
dief	Dieb	thief	lijf	Leib	body
dijk	Teich	pond	lijm	Kleber	glue
doek	Tuch	cloth	lip	Lippe	lip
dorp	Dorf	village	loof	Laub	foliage

melk	Milch	milk	slot	Schloss	lock
mes	Messer	knife	sluis	Schleuse	sluice
mond	Mund	mouth	snor	Schnurrbart	moustache
mug	Mücke	mosquito	soep	Suppe	soup
muts	Mütze	cap	spaak	Speiche	spoke
muur	Mauer	wall	steen	Stein	stone
neef	Neffe	nephew	ster	Stern	star
neus	Nase	nose	stof	Staub	dust
nier	Niere	kidney	stoot	Stoss	push
oog	Auge	eye	strijd	Kampf	battle
pad	Pfad	path	stuur	Lenkrad	steering wheel
piek	Gipfel	peak	taart	Kuchen	pie
pijn	Schmerzen	pain	tand	Zahn	tooth
pijp	Pfeife	pipe	tas	Tasche	bag
pols	Puls	pulse	teek	Zecke	tick
pont	Fähre	ferry	tent	Zelt	tent
prik	Spritze	syringe	tijd	Zeit	time
rek	Regal	rack	tong	Zunge	tongue
rib	Rippe	rib	trap	Treppe	stairs
rijst	Reis	rice	veer	Feder	feather
rit	Fahrt	drive	vis	Fisch	fish
roer	Ruder	rudder	vlees	Fleisch	meat
rug	Rücken	back	voet	Fuss	foot
rups	Raupe	caterpillar	vork	Gabel	fork
sap	Saft	juice	vuil	Schmutz	dirt
schok	Schlag	blow	vuur	Feuer	fire
schol	Scholle	plaice	waard	Wirt	innkeeper
sla	Salat	salad	walm	Qualm	smoke

will	Wille	will
winst	Gewinn	profit
wol	Wolle	wool
wond	Wunde	wound
worst	Wurst	sausage
zalm	Lachs	salmon
zeep	Seife	soap
zit	Sitz	seat
zon	Sonne	sun
zool	Sohle	sole
zout	Salz	salt

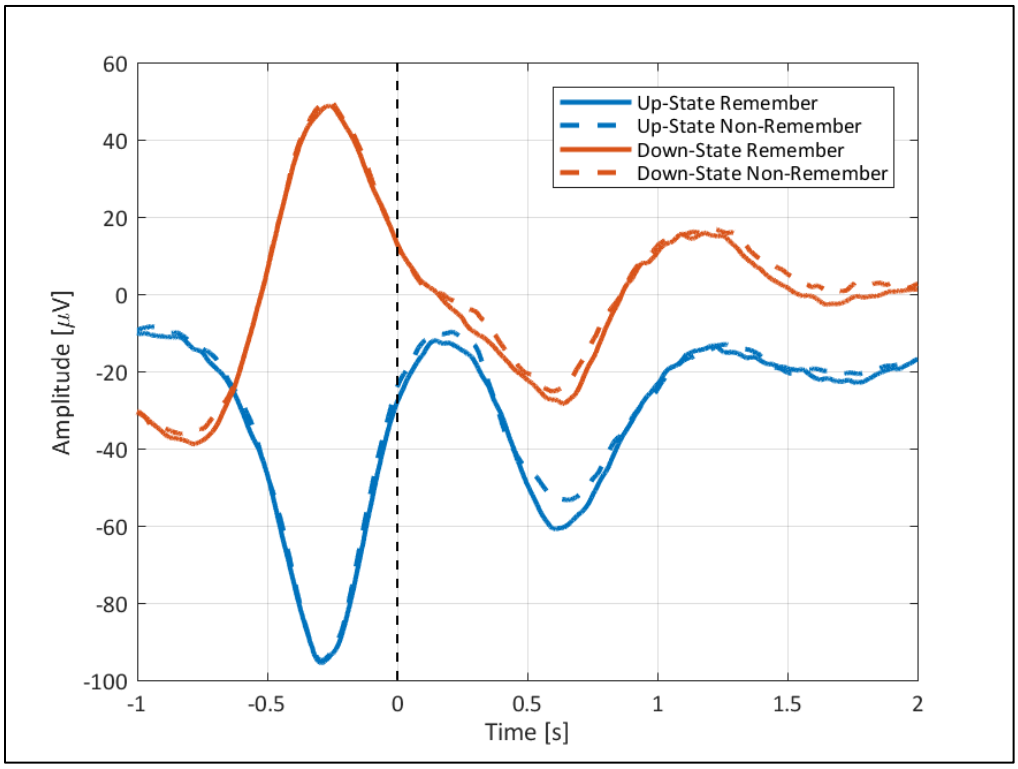
Wordlist used for memory task. Dutch-German word pairs used during the memory task.

Supplementary Figure 1: Slow Wave Detection Algorithm State Diagram



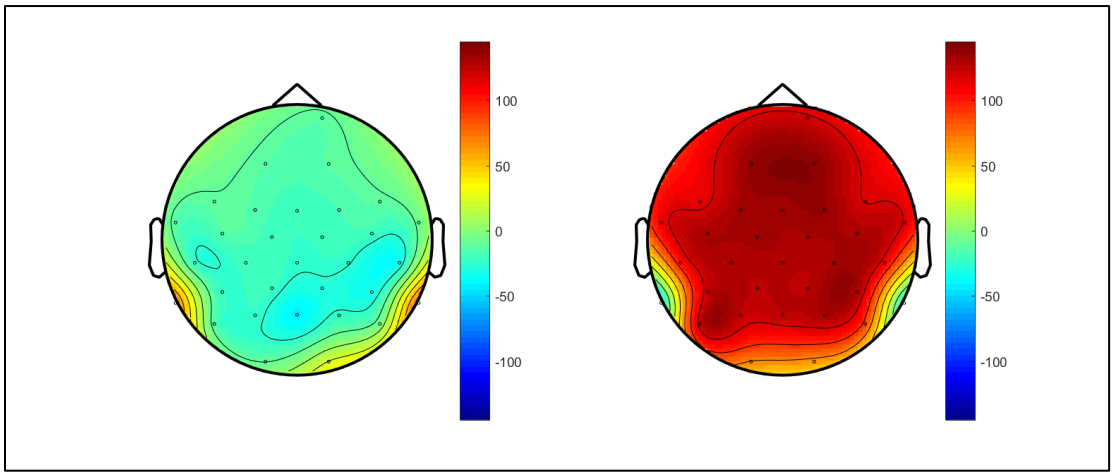
Online Detection Finite-State Machine Diagram Implementation of the slow-wave detection algorithm as a finite state machine. The algorithm starts at the black dot and traverses through the states while it is running.

Supplementary Figure 2: ERPs for Remembered and Non-Remembered cues



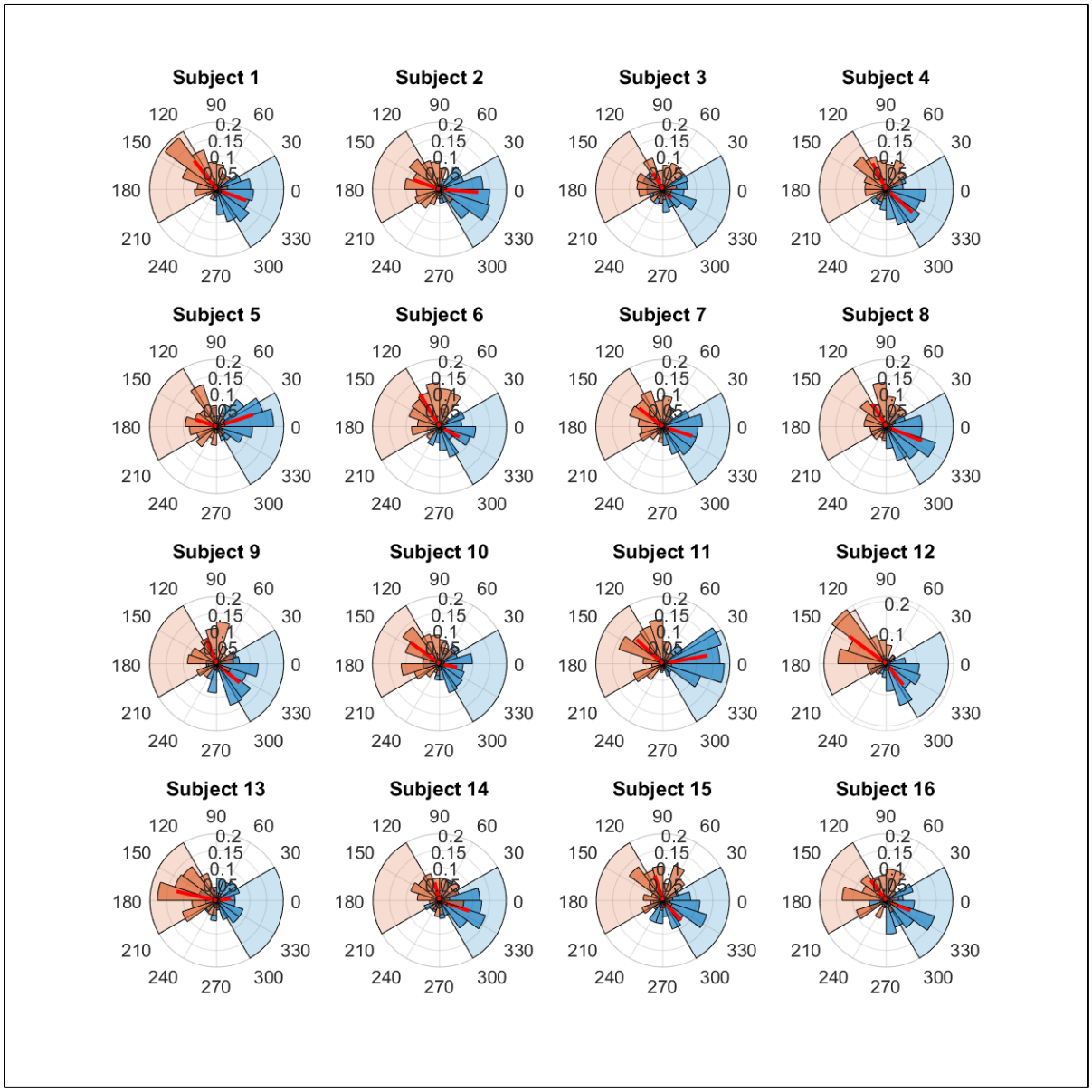
Comparison of ERPs for remembered and non-remembered words. ERPs for up- (blue) and down-state (red) remembered (solid line) and non-remembered (dashed line) words are shown. There is no significant difference between remembered and non-remembered word cues.

Supplementary Figure 3: Phase Distribution across the Scalp



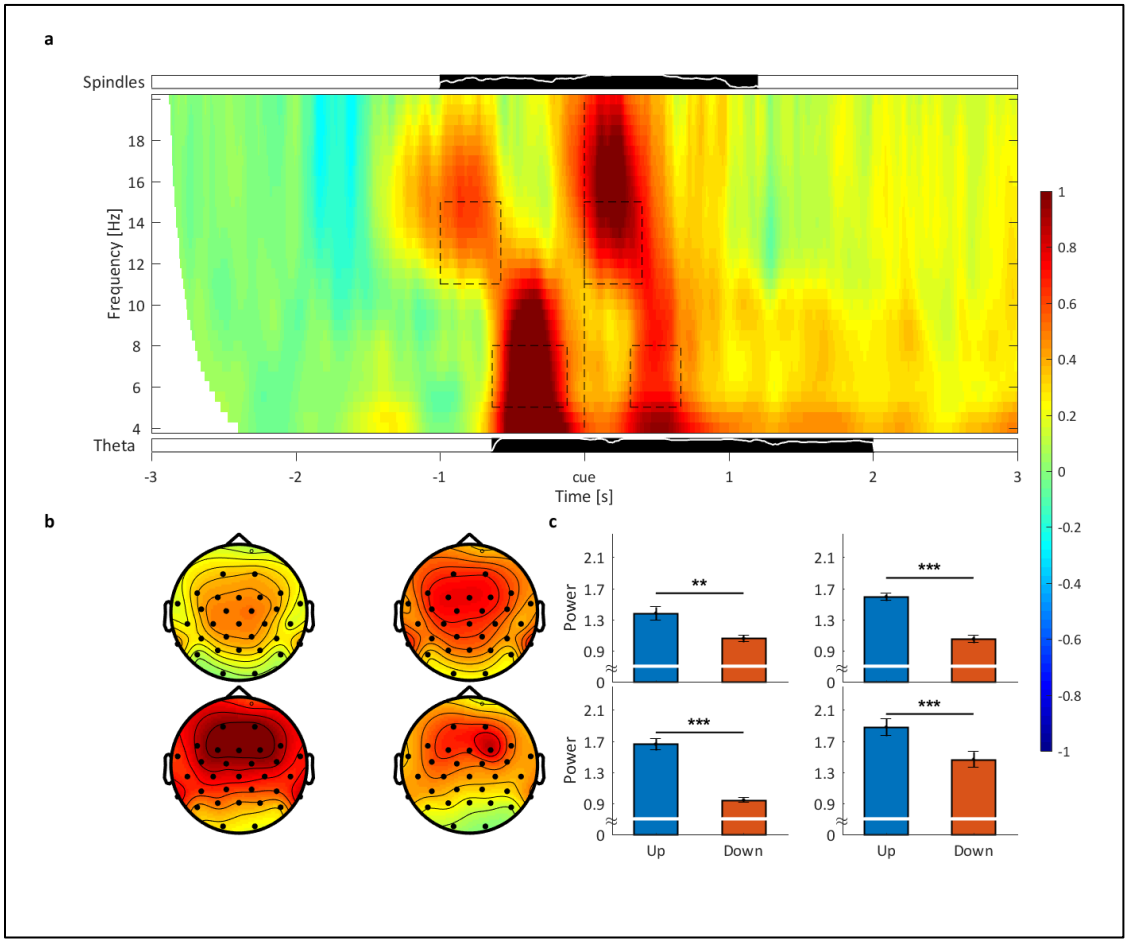
Topographical distribution of phase. Signal phase at stimulus release for up-state cues (left) and down-state cues (right). While the algorithm detects slow-waves at the Fz electrode only, the phase distribution across the scalp is uniform at the time of cue onset. Up-state phase is around -20° and down-state phase around 120°.

Supplementary Figure 4: Phase Accuracy for each Subject



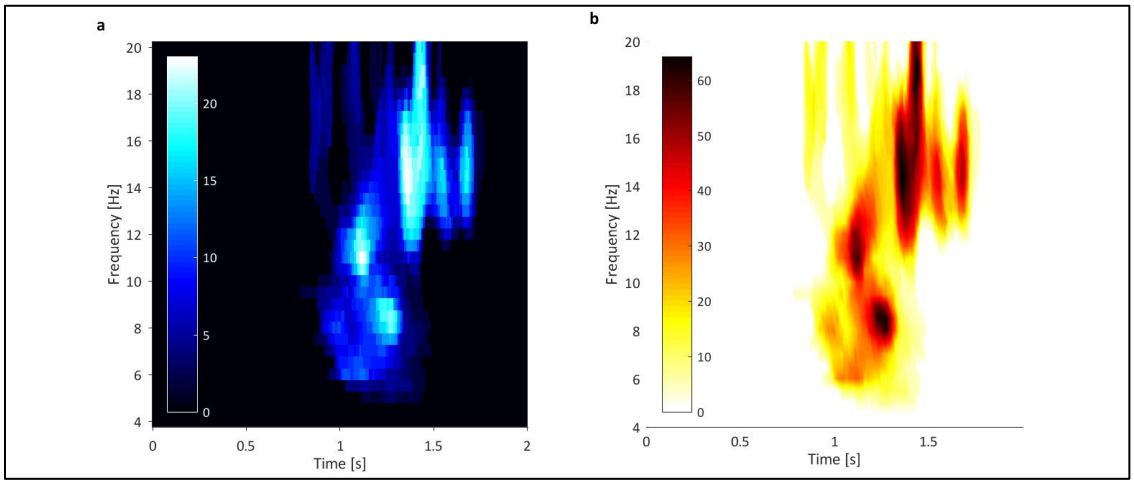
Phase accuracy for each subject at trial level. Up-state cues are shown in blue. Down-state cues are shown in red. *Trial level phase accuracy for each individual subject shows a clear distinction between up- and down-state cues for all subjects.*

Supplementary Figure 5: Oscillatory analysis of up- versus down-state cues.



Oscillatory analysis of up- versus down-state cues. Panel a) illustrates the power contrast between words presented during SOs up- and down-states. Black bars (significant cluster in frequency band analysis) with white lines below and above the time-frequency plot indicate the number of significantly differing electrodes for the theta and spindle band respectively. The full height of the bar corresponds to 100% (31) electrodes. Panel b) topographical distribution of the areas marked with a dashed box in a) for the spindle (top row) and theta (bottom row) band, pre-stimulus (left column) and post-stimulus (right column). Significant electrodes are shown as filled black dots. Panel c) shows the same data averaged across time, frequency and significant channels within the respective cluster. The power is scaled between -1 and 1 for both panels a) and b). Time-frequency data is shown for the Fz electrode.

Supplementary Figure 6: Up-State Remembered versus Non-Remembered Words Time-Frequency Analysis



Positive cluster up-state remembered vs non-remembered. a) Number of electrodes involved in positive cluster found in time-frequency analysis of up-state remembered versus non-remembered words across frequency and time. b) Summed t-values for positive cluster found in time-frequency analysis of up-state remembered versus non-remembered words across frequency and time. The time-frequency analysis of up-state cues of remembered versus non-remembered words between 0 and 2 seconds after stimulus onset, across all channels and from 4 to 20 Hz revealed one significant positive cluster lasting from 0.79 to 1.77 seconds and involving all electrodes (P = 0.016).

3.4 Manuscript IV

No overall memory benefits of unsupervised targeted memory reactivation during sleep at home.

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Candidate contribution:

Maurice Göldi devised, planned, conducted the experiment, analyzed the data and wrote the manuscript with Björn Rasch.

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Abstract

Targeted memory reactivation (TMR) during sleep improves memory consolidation, as has been repeatedly shown in well-controlled studies in the sleep laboratory in recent years. However, it is still unknown whether the memory benefits of TMR can be generalized to real-life conditions. Here we tested whether TMR during sleep enhances Dutch-German vocabulary learning when applied during multiple nights at home in an unsupervised fashion. 66 participants learned and recalled the same list of 120 Dutch-German vocabulary over four days, resulting in a consecutive increase in performance over the multiple active retrieval sessions. During the three intervening nights of sleep, they used an mp3-player to play 60 Dutch words, without any control of sleep or awakenings by tones (unsupervised TMR). In contrast to previous TMR studies in the sleep laboratory, unsupervised TMR during three nights of sleep at home had no effect on retrieval performance on the fourth day. Participants who reported awakenings by tones even showed a selective impairment of words played during sleep (cued words). Participants who did not report awakenings by the auditory stimulation significantly improved memory for cued words across night 3 only. Our results indicate that TMR benefits observed after one night in the sleep laboratory cannot be directly generalized to the home setting. Our findings suggest that habituation as well as automatic sleep monitoring and avoidance of auditory-induced awakenings might be a precondition to successfully apply TMR to language learning in real-life.

Keywords: home-setting, sleep, targeted memory reactivation, vocabulary learning,

In recent years, evidence is accumulating that that re-exposure to memory cues during non-rapid eye movement (NREM) improves later memory performance (e.g., Rasch et al., 2007; Rudoy et al., 2009, Diekelmann et al., 2011). This technique is now referred to as targeted memory reactivation (TMR) during sleep (Oudiette & Paller, 2013). Memory benefits caused by TMR are explained by the assumption that re-exposure to memory cues during NREM sleep increases spontaneous reactivation signals relevant for memory consolidation during sleep (Rasch & Born, 2007). According to the active system consolidation hypothesis (Diekelmann & Born, 2010; Marshall & Born, 2007), spontaneous reactivation of neuronal networks in the hippocampus during NREM sleep are essential for strengthening recently acquired memory traces and their integration into cortical long-term memory stores during sleep (Deuker et al., 2013; O'Neill, Senior, Allen, Huxter, & Csicsvari, 2008; Peigneux et al., 2004; Rothschild, Eban, & Frank, 2016; Wilson & McNaughton, 1994). (Born & Wilhelm, 2012; Rasch & Born, 2013; Schwindel & McNaughton, 2011; Sirota, Csicsvari, Buhl, & Buzsaki, 2003). The additional memory benefit of TMR can be explained by selectively activating specific hippocampal memory traces during sleep through cues, thereby increasing the reactivation of this engram which results in a better consolidated trace (Fuentemilla et al., 2013). The positive effect of TMR during sleep on memory is now well established and has been shown for a variety of memory cues such as sounds (Cairney, Lindsay, Sobczak, Paller, & Gaskell, 2016; Groch, Schreiner, Rasch, Huber, & Wilhelm, 2017; Rudoy, Voss, Westerberg, & Paller, 2009), melodies (Antony, Gobel, O'Hare, Reber, & Paller, 2012; Cousins, El-Deredy, Parkes, Hennies, & Lewis, 2014; Schönauer, Geisler, & Gais, 2014) or verbal material (Lehmann, Schreiner, Seifritz, & Rasch, 2016; Schreiner, Lehmann, & Rasch, 2015; Schreiner & Rasch, 2015). Based on this numerous and robust empirical evidence, it appears obvious to start to apply this technique in real live settings.

However, all studies cited above have conducted TMR under well-controlled laboratory conditions including online sleep monitoring by polysomnography. To our knowledge, no scientific study so far as examined whether the benefits of TMR can be generalized to uncontrolled environment such as a subjects' home, for example by using applications on smart-phones or tablets. Thus, it remains to be shown whether TMR is also successful under unsupervised real-life conditions when factors such as sleep stage, learning level, sound volume or reactivation pausing due to arousal cannot be tightly controlled. Furthermore, all studies so far have tested the effectiveness of TMR after one night of sleep. Again, to our knowledge, there has been no well-controlled study investigating the performance of recent TMR techniques applied over multiple days.

Thus, the aim of this study is to test whether a simple, unsupervised TMR setup applied during sleep improves memory under real-life conditions over multiple days. As a memory task, we used the Dutch-German vocabulary task for which we have repeatedly observed memory improvements by TMR applied during sleep in the lab (Schreiner et al., 2015; Schreiner & Rasch, 2015). Participants trained on the task (120 Dutch-German word-pairs) online over 4 consecutive days. During the three intervening nights in which participants sleep at home in their normal sleeping environment, half of the words (60 Dutch words only) were played (cued) during sleep. The selection of cued words was identical during all three nights of sleep; the other half of the words was never played during sleep (uncued words). For cueing during sleep, participants received an mp3-player containing a sound file starting with 30 min silence and then one hour of repeatedly play Dutch words. They were instructed to play the audio file the following three nights when going to sleep. We hypothesized that words played during sleep (cued words) would be better remembered during retrieval on the fourth day as compared to uncued words. In contrast to our hypothesis, unsupervised TMR over multiple nights had a no positive effect on memory for cued words over the whole experiment period. Detailed analysis revealed that only across the third night, German translations for cued Dutch words were better retrieved then uncued words, particularly in undisturbed sleepers. Participants who reported sound-induced awakenings even showed a selective impairment for cued words at the end of the study procedure.

Materials and Methods

Subjects

78 healthy young subjects completed the study, of which 12 were rejected during pre-processing (see section 'Data Pre-Processing'). 66 participants (45 female) between 18 and 30 (21.86 ± 0.30, mean ± standard error of the mean, s.e.m.) years of age were included in all further analysis. All participants had German or Swiss German as their mother tongue and reported no prior knowledge of Dutch or Afrikaans. Participants had no known sleep disorders (screened by the Pittsburgh Sleep Quality Index (PSQI; Buysse et al., 1989), dyslexia, learning disorders or hearing impairments. The internal review board (IRB) of the University of Fribourg approved the study, and all subjects gave written informed consent prior to participating. Participation was compensated either with 60CHF or university course credits.

Design and Procedure

Participants in this study took part in a vocabulary-learning task, where they were asked to learn 120 German-Dutch word pairs. The experiment included learning and recall sessions on four consecutive days and auditory cueing during the three intervening nights. See Figure 1a top for a schematic overview of the experimental procedure. Additionally, at the beginning of each session participants filled out a questionnaire regarding their previous night's sleep (SF-A/R, (Görtelmeyer, 2011)) plus some additional questions about the comfort of the TMR setup.

On the first day, participants met the examiner in a quiet and undisturbed room between 7pm and 9pm. They received a set of in-ear sports earphones (Philips SHQ3200), which they were instructed to use throughout the experiment during the night and the learning/recall tasks. The experiment was done online using LimeSurvey (LimeSurvey GmbH, Hamburg, Germany). In a first learning round, the participants listened to 120 German-Dutch word pairs. Each Dutch word was presented aurally, and then the written German translation was visible for two seconds in capital letters, followed by a three-second blank screen. After the first round of learning and a short break, participants continued with the second round during which they listen again to all 120 words. During this active recall round, they had to type in the German translation. No feedback was given. Based on recall performance on this first day, 50% of the correctly remembered and 50% of the non-remembered Dutch words (60 words) were randomly selected for cueing during the night (Figure 1a left). A custom script was used to generate an individual audio file for each participant, which was then loaded onto an iPod (iPod shuffle). The participants then used this same recording for three consecutive nights to cue 60 words during their nighttime sleep. Each audio file had a 30-minute silent period at the beginning. Then the words selected for cueing during the night were repeated as a block 13 times with a 5-second inter-word silence, totaling roughly 60 minutes of playback. The first (last) block was presented with linearly increasing (decreasing) volume to minimize waking through sudden sound onset. Participants were instructed to start the audio file when they were in bed and ready to go to sleep. They were also instructed to adjust the volume so that they would hear the words played, but not be disturbed in their sleep. Should they wake up from hearing the words, they were instructed to reduce the volume to a comfortable level and restart the audio file.

On the evening of the second day, participants received an email with a link to the survey at approximately the same time they started the experiment the day before (ca. 7 – 9 pm). They were instructed to continue the experiment at home in a quiet and undisturbed setting using the earphones. The first round was an

active retrieval with feedback of all 120 Dutch-German word pairs. Participants listened to the Dutch word aurally and had to type in the German translation. Then they were presented with the correct German translation on screen for two seconds, regardless of the correctness of their answer, allowing them an opportunity for acquiring more word-pairs correct. After the first round and a short break, the participants did another round of recall, where they listened to the Dutch word and had to type the German translation. No further feedback was given. For the night, the participants were again instructed to sleep using the iPod.

Day 3 followed the same procedure as day 2. On the evening of the final day subjects only had one final recall round without feedback. Over all days the word order was fixed for all participants, but randomized between each learning/recall round.

For each subject, we therefore have a repetition of the same experiment for three consecutive nights. The word pairs tested were the same over the whole experiment and the words cued presented during the night remained identical for each participant. Between the repetitions, participants had an average pre-retention learning level of 37.71%, 52.64% and 62.17% of words remembered for nights 1, 2 and 3 respectively.

Data Pre-Processing

Prior to further analysis all the subjects' typed answers were checked for spelling mistakes. For the German word 'Sonne' (engl. 'sun') the plural form 'Sonnen' or small spelling mistakes (e.g. 'Soonne') were accepted as correct. However, when the word resulted in a word similar to another German word (e.g 'Sohne', where 'Sohn' would be the correct spelling for engl. 'son'), the translation was scored as incorrect. Capitalization was not considered a mistake. This evaluation was done for all given answers by four native German speakers independently, and the results were then aggregated. In some subjects, this procedure unbalanced the number of correct cues chosen for cueing during the night versus uncued words. To avoid any bias, 12 subjects with 4 or more words in one category (cued vs. uncued words) were removed from further analysis, leaving 66 participants.

The recall round without feedback (second round) of every day was used as the baseline performance for the following day. The first round of recall every day was the memory performance after the retention period (night sleep). Memory performance of the active daytime learning on day 2 and 3 was measured using the first recall of the day as a baseline performance and the second recall of the day as recall performance. The percent change of words remembered from baseline to the next recall was computed for

every day. To avoid confusion, we will refer to the different recall time points by the time between baseline and recall measurement (i.e. the respective retention interval). 'night 1', 'night 2' and 'night 3' will refer to the first recall on day 2, 3 and 4 respectively. The learning interval from the first recall with feedback to the second recall without feedback on day 2 and 3 will be referred to as 'day2' and 'day3'. Words that were remembered during the recall were termed as 'Remember'. Words that were not remembered during recall were termed as 'Non-remember'. For a more fine-grained analysis we further divided our behavioral data into the sub groups 'gain', 'loss', 'hithit', 'missmiss'. Gains are words that are not remembered at baseline, but remembered at recall. Losses are words that are remembered at baseline, but not remembered at recall. Hithits are words that are remembered at both baseline and recall. Missmisses are remembered neither at baseline nor recall. Note that the remember group contains the gain and hithit words and the non-remember group contains the loss and missmiss words. We therefore focus only on the gain and loss groups, as the hithit and missmiss groups would give complementary results. See Figure 1a left for an overview of word groupings.

Statistical Analysis

Data was analyzed using repeated-measures analysis of variance (ANOVA) with the within subject factors time and word type (cued vs uncued). For analysis where the behavioral data was further subdivided, we used an additional factor memory outcome (gain, loss). Post-hoc pair wise comparisons were done using two-sided paired t-tests. Statistical significance threshold was set to $P = 0.05$. Mean values are reported as mean ± s.e.m. All analyses were done using Matlab (The Math Works Inc., Natick, MA, USA).

Results

Effects of unsupervised TMR at home on sleep quality

As expected, presentation of acoustic cues with the headband resulted in a decrease in subjective sleep quality (scaled 0 to 4) in the first and second night: Participants rated their subjective sleep quality significantly lower in the first experimental night (2.23± 0.12) as compared to the night without tone stimulation (3.04 ± 0.08; $t(65) = 5.70$, $P < 0.001$). Sleep quality in night 2 was still significantly reduced (2.65 ± 0.01; $t(65) = 2.86$, $P = 0.006$), while sleep quality in night 3 did not differ from sleeping without sound stimulation (2.85 ± 0.08; $P > 0.10$). Overall, a linear trend of increasing sleep quality across the 3 experi-

mental nights was observed ($F(1,65) = 30.29$, $P < 0.001$; $\eta 2 = 0.32$). More specifically, 19 participants reported awakenings and disturbed sleep due to the repeated tone stimulation during the first night (15 in the second, 5 in the third night). In contrast, we observed no significant differences in subjectively reported sleep duration (experimental nights: 8.07 ± 0.14, hours, 7.71 ± 0.15 hours and 7.89 ± 0.13 hours, respectively, control night: 7.86 ± 0.15 hours, all $P > 0.19$).

Overall memory performance

Overall, participants successfully recalled 45.26 ± 1.57 German translations of the newly learned Dutch words (37.72% of all 120 word-pairs) before night 1. Four days later at the end of the study procedure they recalled on average 72.58 ± 2.40 words, resulting in a clear improvement of 27.32 ± 1.76 words (22.77%) over the multiple learning sessions ($t_{(65)} = 15.52$, $P < 0.001$). The improvement was almost exclusively due to active rehearsal on day 2 and 3 (both $P < 0.001$; see Figure 1b). In contrast, memory performance over nights 1, 2 and 3 remained stable relative to their preceding levels in spite of TMR (all $P > 0.05$; see the table in Figure 1a for an overview of the number of words in each word category and at each measurement point).

Next, we analyzed memory improvements separately for words played (cued) or not played (uncued) during sleep in the three nights of the experiment. In contrast to previous results obtained in the sleep laboratory after one night of sound stimulation, we did not observe any general memory benefit of targeted memory reactivation (TMR) during sleep at home. For cued words, participants increased their memory performance from day 1 to day 4 by 163.42 ± 4.66%, while uncued words that were never played during sleep improved by 167.29 ± 5.61%. We observed no significant difference in memory improvement between cued and uncued words ($t_{(65)} = 1.14$, $P = 0.257$). Number of cued and uncued words did not differ at baseline ($t_{(65)} = 1.58$, $P > 0.12$). Thus, our overall analysis reveals that cueing of Dutch vocabulary during three nights of sleep at home does not generally benefit memory.

One major difference to previous studies in the sleep laboratory was the unsupervised word presentation during sleep. Thus, the auditory stimulation was not immediately stopped by the experimenter when any signs of awakenings occurred. During the first night, n = 19 of the 66 participants reported awakenings due to tone presentation during sleep. To control for the effects of awakenings by the tone stimulation, we used the reports from the first night to separate participants in disturbed and undisturbed sleepers as additional factor in our analysis. We observed a highly significant interaction between cueing success and

tone-induced awakenings ($F_{(1,64)}$ = 7.11, P = 0.01, η^2 = 0.10): Over the three nights of stimulation, undisturbed sleeper descriptively showed even a slight, but non-significant memory improvement by TMR (cued words: 166.89 ± 5.20% vs. uncued: 165.27 ± 5.78%; P > 0.60, see Figure 1c). In contrast, disturbed sleepers overall exhibited a significant impairment by TMR during three nights of sleep by ca. 17% (cued: 154.84 ± 9.78% vs. uncued: 172.30 ± 13.49%, $t_{(18)}$ = 2.20, P = 0.041). Thus, awaking by TMR appears to have an impairing effect on memory consolidation during sleep. Importantly, this impairment seems to be selective for those words presented during sleep, but not for uncued words. Due to the significant interaction of memory performance with auditory-induced awakenings, we present all subsequent results on memory performance also separately for undisturbed and disturbed sleepers.

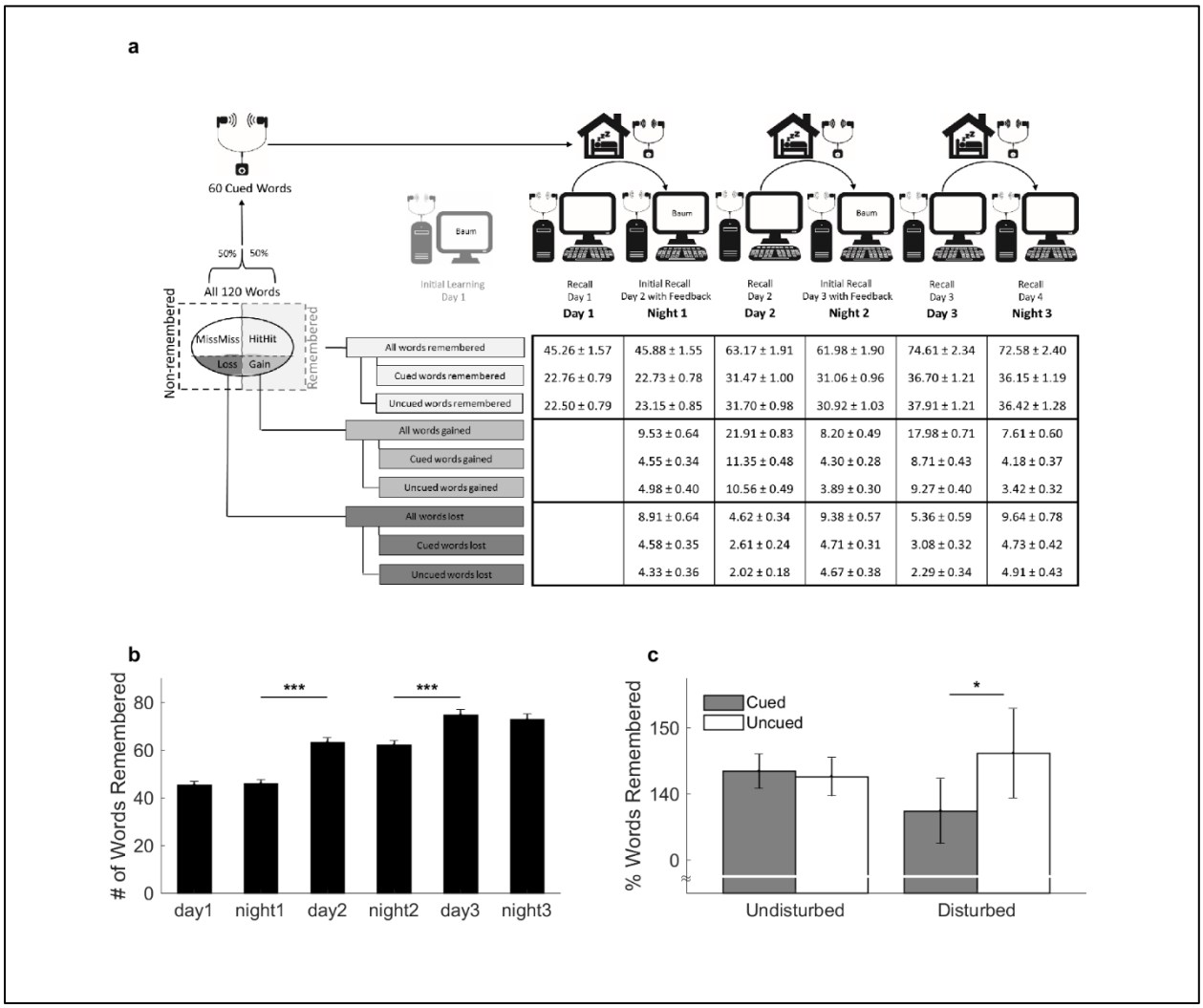

Figure 1|Results overview. a) A schematic overview of the experimental procedure (top), data grouping for analysis (left) and results (table). Participants perform a Dutch- German word learning task in the evening on four consecutive days. During the nights between experimental days, participants sleep at home with an mp3-player listening to verbal cues of the learned vocabulary. On each day, participants had two task rounds. In the first round, all Dutch words were presented aurally, then participants

typed in the German translation (except for initial learning on day 1, where no response had to be given). This was followed by the correct written German translation on screen (except on day four, where no feedback was given). During the second round before sleep, participants heard the Dutch word and then gave their translation. No further feedback was given. The 120 Dutch-German word pairs were grouped into remembered and non-remembered words after the second round on day 1. 60 Dutch words (50% of the remembered and 50% of the non-remembered words) were randomly selected and put on an mp3-player for TMR during the night. Only Dutch words were played during sleep, without the German translations. Memory performance was measured as words remembered at each point of measurement (results in top three rows of table). Further analysis showed words gained (rows 4 to 6) and words lost (rows 7 to 9) relative to the previous measurement point. The table shows number of words for all, cued and uncued words of each category. See Supplementary Table 1 for percent changes. b) Number of words remembered over time. The total number of words remembered grows over time. There is an increase during daytime learning and a stabilization during nighttime retention. c) Percent words remembered across the complete experiment for undisturbed (left) and disturbed (right) sleepers. The percentage of words remembered on the final recall of the experiment after night 3 relative to the number of words remembered on day 1. There is an increase in words remembered. For undisturbed sleepers, cued words during sleep were not better remembered than uncued words. Surprisingly, disturbed sleepers remembered uncued words better than cued words. *: $P \leq 0.05$, ***: $P \leq 0.001$; means ± standard error of the mean (s.e.m.) are indicated.

Effects of TMR on memory across single nights

As the majority of sleep laboratory studies examined memory effects of TMR after a single sleep episode, we additionally analyzed the effects of TMR on memory for each night separately. For this analysis, memory performance before each night was taken as baseline and set to 100% as in previous studies. In an overall ANOVA using the within-subject factors cueing (cued vs. uncued) and night (night 1, 2, 3), we observed a significant interaction between these factors ($F_{(2,130)} = 4.01$, $P = 0.03$; $\eta^2 = 0.06$), confirming that TMR had differential effects on memory for the three nights. In the whole sample, cued words significantly profited from TMR during sleep only in night 3 and were significantly better remembered (99.95 ± 1.96%) than uncued words (96.29 ± 1.53%; $t_{(65)} = 2.32$, $P = 0.024$, not shown). This benefit of TMR was even more pronounced in night 3 when restricting the analyses to undisturbed sleepers, revealing a memory benefit by TMR by ca. 7.7% points (cued words: 102.10 ± 2.29% vs. uncued: 96.37 ± 1.70%; $t_{(46)} = 2.96$, $P = 0.005$, see Figure 2a). In contrast, disturbed sleepers exhibited the opposite result pattern (cued: 94.65 ± 3.60% vs. uncued: 96.11 ± 3.36%. $P > 0.50$, See Figure 2b). The interaction between the factors cueing and tone-induced awakenings was significant in night 3 ($F_{(1,64)} = 4.46$, $P = 0.039$; $\eta^2 = 0.065$).

Descriptively, cued words also profited from TMR during night 2, although the difference did not reach significance, neither in the whole nor in the separate samples (all $P > 0.30$). The result pattern was similar in undisturbed and disturbed sleepers in night 2 (interaction effect $P > 0.90$).

In the first night, an impairment of memory by cueing appeared on the descriptive level in the whole sample, and this impairment was close to a statistical trend ($t_{(65)} = 1.50$, $P = 0.13$). Participants disturbed by tone-induced awakenings exhibited even an impairment of ca. 8.5% points by cueing in the first night: Disturbed sleepers correctly recalled 106.98 ± 5.53% of the words not played during sleep (uncued),

whereas memory performance dropped to 98.17 ± 4.38% for cued words ($t_{(18)}$ = 1.89, P = 0.075). In contrast in undisturbed sleepers, the impairment by cueing was quite small (ca. 1.7% points; P > 0.50). The interaction was not significant in night 1 ($F_{(1,64)}$ = 1.72, P = 0.19, η^2 = 0.26), while the main effect of a general impairment by cueing in the first night almost reached significance in this analysis ($F_{(1,64)}$ = 3.69, P = 0.059, η^2 = 0.055).

The same overall result pattern emerged when analyzing gained words (words that were not remembered before, but recalled after sleep). In night 3, TMR during sleep resulted in a higher percentage of gains for cued words (6.97 ± 0.61%) than uncued words (5.70 ± 0.52%; $t_{(65)}$ = 2.28, P = 0.026; all other nights P > 0.20, not shown). In undisturbed sleepers, the difference was even more pronounced in night 3 (cued gains: 7.23 ± 0.78% vs. uncued gains: 5.50 ± 0.61%; t(46) = 2.60, P = 0.012) and almost reached a trend in night 2 (cued gains: 7.30 ± 5.66% vs. uncued gains: 6.35 ± 5.61%, t(46) = 1.65, P = 0.105). No differences occurred in the first night (P > 0.50, See Figure 2c). In disturbed sleepers, no significant differences between cued and uncued gains occurred on any night (all P > 0.20. see Figure 2d). The percentage of lost words did not differ between cued and uncued words on any of the nights, neither in the whole sample (all P > 0.40) nor for undisturbed sleepers (all P > 0.30). For disturbed sleepers in night 1, cued losses (7.98 ± 8.31%) were marginally higher than losses for uncued words (6.84 ± 7.85%, t(18) = 1.95, P = 0.067). No differences occurred in night 2 and 3 (both P > 0.30).

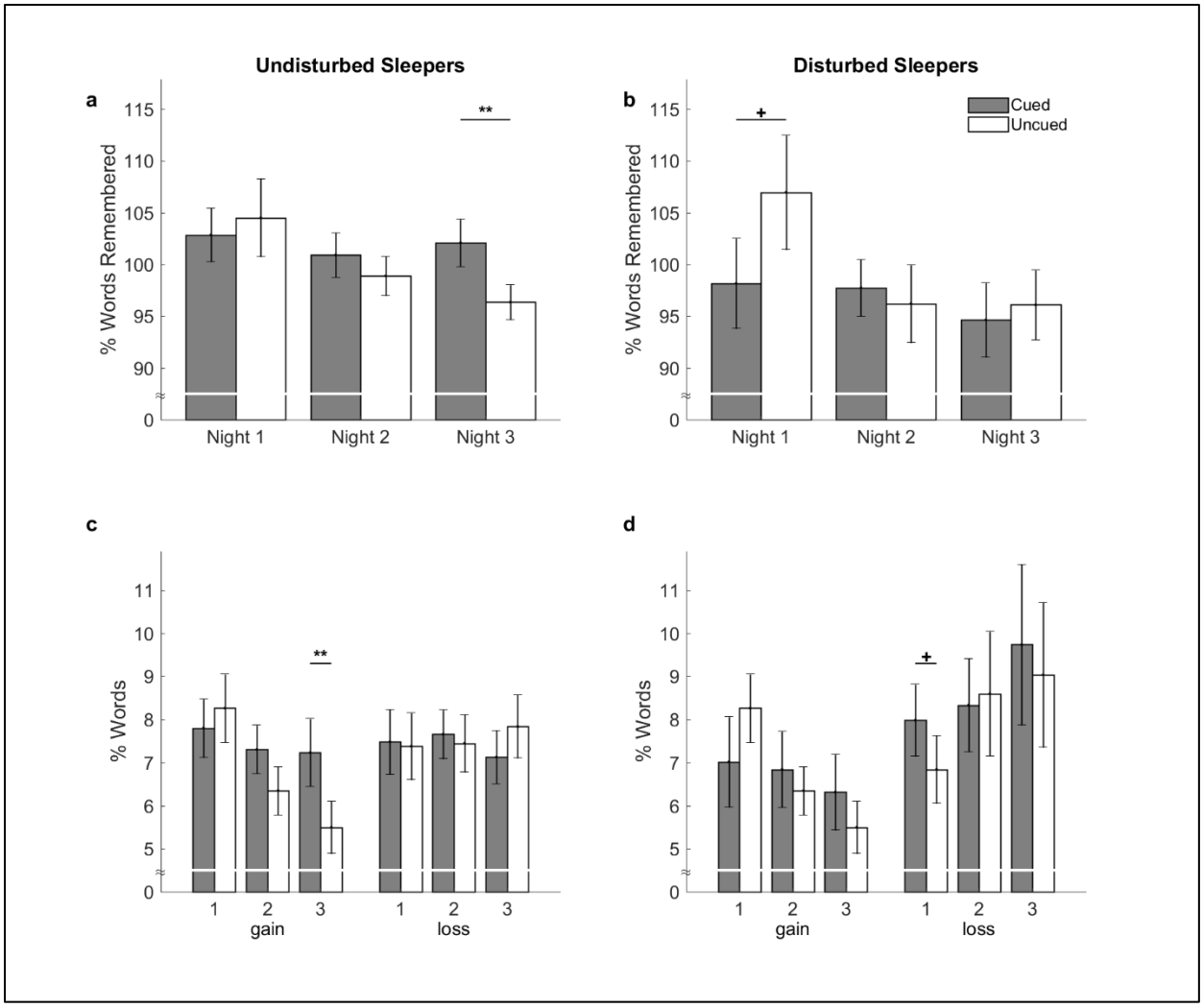

Figure 2| Over-night memory performance (performance before each night set to 100%)**.** a) Undisturbed sleepers: Only in night 3, over-night memory performance was significantly higher for cued words (white) as compared to uncued words (gray). No difference between cued and uncued words occurred across the first or second night. b) Disturbed sleepers: There were no significant differences between cued and uncued words during any of the nights. During night 1 there is a statistical trend (P = 0.075) for uncued words to be better remembered than cued words. Percent words gained and lost for c) undisturbed and d) disturbed sleepers. There was only a difference between cued and uncued gained words during night 3 for undisturbed sleepers, with more cued words gained. +: P ≤ 0.075, **: P ≤ 0.01; means ± s.e.m. are indicated.

TMR across single nights and prior learning levels

Previous studies have shown that the benefit of TMR during sleep on memory are stronger with higher prior learning levels (Creery, Oudiette, Antony, & Paller, 2015) or prior knowledge (Groch et al., 2017). As prior learning levels were lower before the first night as compared to the third night (45.26 1/- 1.57 vs. 74.61 ±2.35 remembered words (of 120), t(65) = 15.30; P < 0.001), one might argue that the selective benefits of TMR in night 3 is due to differences in prior learning levels. To examine this notion, we matched

16 participants of night 1 with 16 different participants of night 3 based on their learning performance before night 1 and 3, respectively. Only participants that did not report any awakenings by the audio stimulation were considered for the matching procedure. After matching, both groups of participants had the same prior learning levels (Night 1 group: 57.12 ± 2.21 words vs. Night 3 group: 57.00 ± 2.43 words, P > 0.97). However, in spite of the equalized prior performance levels before night 1 and 3, the overall result pattern reported above for the whole sample of undisturbed sleepers did not change: Only the night 3 group of participants exhibited a significant TMR benefit on memory of 10% (Cued words: 105.82 ± 5.14% vs. uncued: 95.84 ± 4.25%, t(15) = 3.62, P = 0.003). In contrast, the night 1 group showed no significant memory benefit of TMR occurred (cued words: 92.22 ± 3.39% vs. uncued: 91.90 ± 4.59%; P > 0.90). The interaction between the factors cueing and group (night 1 vs. 3) was highly significant in the corresponding ANOVA ($F_{(1,30)}$ = 6.42, P = 0.017, η^2= 0.18), with an additional significant main effect of a general benefit of TMR on memory (F(1,30) = 7.30, P = 0.011, η^2 = 0.20). Thus, differences in prior learning levels between night 1 and 3 cannot account for the specific TMR benefits after night 3.

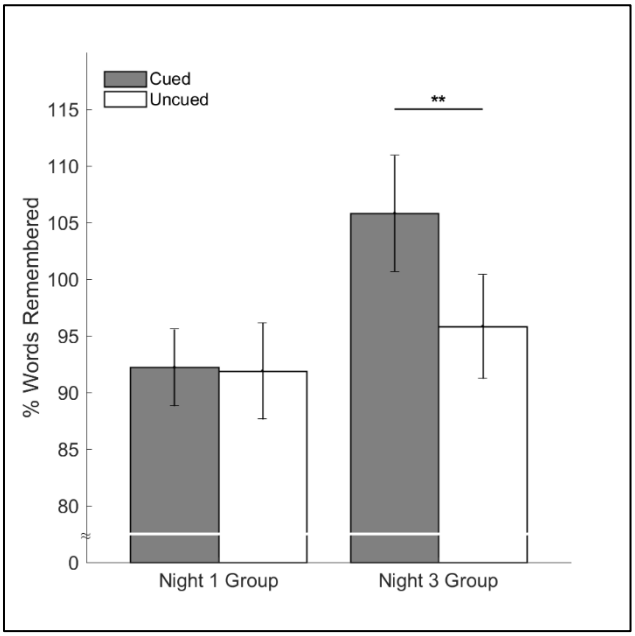

Figure 3| Over-night memory performance of undisturbed sleepers with matched learning levels for night 1 and 3. In groups matched by prior learning level there was no difference between cued and uncued words during night 1. After night 3 however, there were more cued than uncued words remembered. ** : p ≤ 0.01; Means ± s.e.m. are indicated.

Memory performance during active rehearsal during the day

In addition to the memory retention overnight, we used a similar approach to analyze changes in memory performance across the two learning rounds during the experimental days 2 and 3. Thus, the first recall after sleep was taken as baseline and set to 100%. During this recall, participants received feedback for each word on the correct German translation regardless whether it was correctly recalled or not. Then they were tested immediately afterwards again without any feedback. Overall, we observed a main effect of time (day 2 vs. day 3), with higher relative improvements during active rehearsal on day 2 as compared to day 3 ($F_{(1,65)} = 30.16$, $P < 0.001$, $\eta^2 = 0.32$; see Figure 1a table for absolute numbers of correctly recalled words). No other main effects or interactions were observed (all $P > 0.18$).

Discussion

In this study, it was tested for the first time, whether unsupervised TMR works in an uncontrolled environment over multiple days. We have shown that cueing vocabulary of a foreign vocabulary-learning task during the night while sleeping at home shows no memory benefit over a three-night reactivation period. Only across night 3, reactivation of Dutch words during sleep improved memory for the German translation, similar to previous studies in the sleep laboratory.

Due to the unsupervised presentation of word stimuli during sleep, several participants reported awakenings by auditory presentation during sleep. Participants who reported auditory-induced awakenings exhibited a selective memory impairment of words played during sleep after the entire experimental procedure. This impairment was most pronounced after the second night during which words were played for the first time. In undisturbed sleepers, the improvement by TMR across night 3 was greater than in the whole sample. However, we did not observe significant improvements by TMR were observed across night 1 and 2 as well as no overall improved retrieval of cued words at the end of the experiment.

The failure of finding an improving effect of a well-established procedure like TMR during sleep in the home setting is astonishing. Furthermore, we expected that the positive effect of TMR on memory reported after one night would be greater or even additive if memories were reactivated during multiple nights in a row. While cannot provide any definite explanation of the lack of TMR effect in our study, we

can try to examine the most obvious differences between our study at home and previous studies in the sleep laboratory.

The first major difference is that we used an unsupervised auditory stimulation method to present words during sleep. Due to reasons of simplicity and external validity, no automatic sleep monitoring was performed in the current study. Participants themselves simply pressed start on their mp3 player when they went to sleep. The audio file then first has a half hour of silence before the cues are played for 60 minutes. These 90 minutes correspond to the typical time before the onset of REM sleep in the first sleep cycle of the night (Kyriacou & Hastings, 2010). When participants woke up, they either continued listening to the words until the fell asleep again. Otherwise, they stopped the presentation and started it again. Thus, words played during sleep but possibly also wakening. In addition, words could have been played during all sleep stages, including N1 or REM sleep. In contrast in the sleep laboratory, Word presentation is typically started when stable N2 or even N3 sleep occurs. Most importantly, sleep and auditory stimulation are continuously monitored by the experimenter, and the stimulation is immediately stopped when any signs of awakenings occurred.

Interestingly, participants who reported auditory-induced awakenings even exhibited an impairing effect of TMR during sleep at home, at this impairment was most pronounced in the first night with auditory stimulation. Importantly, memory was only impaired for those words actually played during sleep, while memory for uncued words was unaffected. Thus, it is unlikely that the awakenings generally disturbed consolidation processes during sleep, because in this case also memory for uncued words should have been affected. Interestingly, we have also found this negative cueing effect in a previous study using TMR with mp3 players at home (Masterthesis A. Gomez) and during the piloting phase of the current study. Forgetting processes after reactivation cues have been widely discussed in the context of reconsolidation processes (e.g. Nader & Hardt, Nat Rev Neurosci, 2009; Lee JLC, Nader K, Schiller D., Trends Cogn Sci, 2017). According to this account, reactivating a memory renders the trace again in an active and instable state, which is susceptible to forgetting in the presence of interference. One could speculate that awakening immediately after memory reactivation during sleep represent this type of interference of ongoing reconsolidation processes, thereby leading to a selective forgetting of cued as compared to uncued words (see also Diekelmann et al., 2010, for a similar argument). However, we do not have enough data to specifically examine this question, as we do not know when exactly the participants were awakened and what word was played at that time. Future studies are necessary to examine this issue by directly comparing reactivation of words below and above the awakening threshold during sleep.

But also in undisturbed sleepers, we did not observe any general benefit of unsupervised TMR at home on memory for Dutch-German vocabulary. Only in night 3, the typical TMR memory benefit reached significance. One possible explanation is that participants needed to generally adapt and habituate to the auditory stimulation procedure including sleeping with the head band. Subjective sleep quality only reached normal baseline levels in the third night, which coincides with the TMR benefit on memory. Also here, future studies are needed to more systematically examine the relationship between sleep quality and TMR benefits. An alternative explanation could be that TMR benefits on memory require a certain level of prior learning levels or prior knowledge. (Creery, Oudiette, Antony, & Paller, 2015) reported that TMR only benefited memory in participants with high prior learning performance, whereas no TMR effect was observed in low performing participants. TMR during sleep was also ineffective in participants with perfect baseline levels. In addition, (Groch et al., 2017) showed that pre-existing knowledge on to-be-learned objects was require for a TMR benefit on memory, while no TMR effect for learning completely unfamiliar object-name associations. As prior learning levels were quite low before night 1 (37%) and consecutively increase across the multiple active retrieval events in our study, one might speculate the learning performance only reached a sufficient level on Night 3 to allow for a significant TMR benefit on memory. We aimed at excluding this interpretation by selecting participants from night 1 and 3 and matching them according to their learning level before the respective night. Still, we observed a TMR benefit selectively for night 3, rendering the explanation of prior learning levels for our reported effects rather unlikely. However, we cannot completely exclude that the amount of prior knowledge might have still played a role for our results, as prior learning levels might not be the ideal indicator of prior knowledge or even learning capabilities in general.

In sum, we have to conclude that unsupervised TMR during sleep does not generally improve memory for Dutch-German word pairs of three nights of stimulation in a home setting. While we find some promising hints for memory improvements by TMR in night 3, future studies will have to test whether this improvement is actually still present when more nights are examined. Furthermore, our results strongly suggest that also in home settings, automatic sleep monitoring and avoidance of auditory-induced awakening might be crucial to apply TMR benefits on memory in real-life.

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Conflict of Interest: None declared

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Supplementary

3.5 Percent words overview

	day1	night1	day2	night2	day3	night3
% All remember		103.32 ± 2.40	140.28 ± 2.84	98.84 ± 1.45	120.85 ± 1.86	97.85 ± 1.55
% Cued remember		101.51 ± 2.23	140.93 ± 3.03	100.00 ± 1.72	118.61 ± 2.18	99.95 ± 1.96
% Uncued remember		105.22 ± 3.10	140.67 ± 3.99	98.12 ± 1.72	120.92 ± 2.92	96.29 ± 1.53
% All gain		7.94 ± 0.53	18.26 ± 0.69	6.83 ± 0.41	14.99 ± 0.59	6.34 ± 0.50
% Cued gain		7.58 ± 0.57	18.91 ± 0.80	7.17 ± 0.47	14.52 ± 0.72	6.97 ± 0.61
% Uncued gain		8.31 ± 0.66	17.60 ± 0.82	6.49 ± 0.51	15.45 ± 0.67	5.71 ± 0.53
% All loss		7.42 ± 0.54	3.85 ± 0.28	7.82 ± 0.48	4.47 ± 0.49	8.03 ± 0.65
% Cued loss		7.63 ± 0.59	4.34 ± 0.39	7.85 ± 0.51	5.13 ± 0.53	7.88 ± 0.70
% Uncued loss		7.22 ± 0.59	3.36 ± 0.30	7.78 ± 0.63	3.81 ± 0.56	8.18 ± 0.71

Supplementary Table 1| Percent words for all word groups and all measurement points. Measurement points and word groups correspond to the table in Figure 1a. Percent values relative to the previous measurement point are shown. Results are mean ± s.e.m.

4 Discussion

This thesis presents four manuscripts related to the study of oscillatory mechanisms underlying memory consolidation during sleep. This section will discuss the links between the individual manuscripts and it will be shown how they build on each other. Additionally, some open research questions that arise in the context of the presented manuscripts will be put forward and discussed. Finally, the findings will be discussed in context of the two eminent hypotheses of sleep function, ASH and SHY. The goal is threefold: Mainly, discuss the different oscillatory mechanisms as they are understood today and how they relate to memory consolidation during sleep; propose and discuss speculative models that may explain some of the open research questions; and finally, present a first practical implementation for an every-day TMR scheme.

4.1 The Critical Role of Theta Activity for Memory Consolidation

Manuscript I addresses the question of whether targeted memory reactivation during sleep enhances subsequent retrieval related neural oscillations. It was previously shown that TMR of foreign vocabulary during sleep enhances memory performance on subsequent recall performance (Schreiner & Rasch, 2015a). However, these enhancements were only reported on the behavioral level (i.e. the number of words recalled). It remains to be shown that TMR can induce changes that are reflected in the oscillatory response to a cue during wake. Indeed, we find that theta activity, previously implicated in memory encoding and retrieval, is enhanced in post-sleep recognition testing for words that were cued during sleep. Additionally, we find that irrespective of cueing, previously learned words show an increase in theta and gamma band activity while new words do not. We were even able to localize the theta band activity in source space to the left inferior prefrontal cortex (Brodmann areas 45 and 47), previously associated with deep (versus shallow) encoding of words. An important point to note is that behaviorally TMR only had an effect on word recall, but not on word recognition. Theta activity seems to be a more sensitive measure of memory recognition than behavioral outcome. Manuscript I therefore is able to confirm previous research that shows theta activity to be an important marker for successful memory retrieval (Bakker, Takashima, van Hell, Janzen, & McQueen, 2015; Jacobs, Hwang, Curran, & Kahana, 2006; W. Klimesch et al., 2006; Nyhus & Curran, 2010).

Additionally, the results add support to the ASH by showing that TMR during sleep also leads to persisting changes in neural oscillations during subsequent wake. It remains an open research question if and how these oscillatory changes persist over time. The relevance of manuscript I in the context of this thesis is,

that it underlines the importance of theta activity to memory processes in the brain during wake and links them to reactivation during sleep. Findings in other research (Groch et al., 2017; Lehmann et al., 2016; Schreiner, Lehmann, et al., 2015; Schreiner & Rasch, 2015a), and confirmed in manuscript III (Göldi et al., 2017), show that theta activity is associated with successful memory reactivation during sleep. This supports the notion that, although not typically associated with sleep, theta activity plays a crucial role in a crucial function of sleep: memory consolidation.

4.2 The Interplay of Slow Oscillations and Sleep Spindles

An oscillatory event typically associated with sleep is slow wave activity. With its large amplitude it is a very prominent feature measured in surface EEG. It involves large populations of neurons and it is thought to synchronize the brain across multiple regions. Both ASH and SHY ascribe SWA, and specifically SOs, a major pace giving role in their hypotheses. It remains to be shown which hypothesis captures the function of SOs better, or if both mechanism can be active at the same time.

SWA is an important marker for sleep pressure (A A Borbély, 1982). It decreases overnight and has been linked the restorative processes of deep sleep (Tononi & Cirelli, 2003, 2006). Huber and colleagues (2004) showed for the first time in humans that local changes in sleep intensity, as measured by SWA, are driven by learning. This learning is achieved by spontaneous reactivation of memory traces during slow oscillations to strengthen synaptic connections (according to ASH (Diekelmann & Born, 2010)), or by protection of strong synaptic connections from global downscaling (according to SHY (Tononi & Cirelli, 2014)). Irrespective of the underlying mechanism it is clear that SOs are positively linked to memory consolidation processes during sleep. A potential possibility to enhance memory consolidation during the night is to increase the number of SOs. In other words, enhancing memory performance by enhancing the environment within which consolidation is thought to take place.

How can the number of SOs be increased during sleep? Acoustic stimulation during sleep has been identified as a non-invasive, safe and simple way to enhance sleep slow waves (Bellesi et al., 2014). The beneficial effect of acoustic stimulation on memory consolidation has been shown in (Ngo et al., 2013). However, enhancing SOs does not guarantee an improvement of memory performance (Weigenand et al., 2016). Manuscript II (Göldi & Schreiner, 2017) is a commentary on the open-loop acoustic stimulation paradigm implemented by Weigenand and colleagues (Weigenand et al., 2016). It contrasts the work to previous studies implementing a closed-loop acoustic stimulation approach (Ngo et al., 2013). Important conclusions are drawn with respect to nested neural oscillations, the importance of endogenous brain rhythms and their relation to memory consolidation. A memory improvement is only observable with

closed-loop (i.e. playing a tone into the up-state of a endogenous SO) acoustic stimulation (Ngo et al., 2013). Open-loop (i.e. playing a tone to induce a SO and then playing another tone into its up-state) acoustic stimulation on the other hand does not lead to memory improvements (Weigenand et al., 2016). This suggests that it is only the endogenous SOs that lead to an enhancement in memory consolidation. In this case, forcing the brain into an oscillatory pattern does not produce beneficial consolidation effects. The ASH proposes that it is not only the SO that is important for memory consolidation, but rather the interplay of different oscillatory events. Specifically, SOs nest sleep spindles, which in turn nest SWRs. The findings discussed in manuscript II would therefore support at least the functional link between SOs and sleep spindles. SHY on the other hand does not propose a specific mechanism (or even claim an involvement (Tononi & Cirelli, 2012)) of spindles in the down-scaling of synaptic strength. Therefore, the findings in no way conflict with SHY, at the same time SHY cannot offer a mechanistic explanation at this point. The aforementioned nesting has been confirmed experimentally (Staresina et al., 2015) in humans. Manuscript III (Göldi et al., 2017) as well as the preceding experiments (Lehmann et al., 2016; Schreiner, Lehmann, et al., 2015; Schreiner & Rasch, 2015a) have also established a strong link between SOs and spindle activity. Indeed, there is an increase in sleep spindle activity in the closed-loop paradigm and a decrease of sleep spindle activity in the closed-loop paradigm. It seems like the endogenous SO was able to induce spindle activity, while the induced SO was not. It is not yet fully understood how the interaction between SOs and sleep spindles work, and therefore why closed-loop stimulation is able to induce sleep spindles, while open-loop stimulation is not.

Following I present a possible model to explain the difference between endogenous and induced SOs with respect to spindle generation. Figure 6 shows schematic representations of possible interaction mechanisms between SOs and sleep spindles. An endogenous process P could be responsible for evoking a SO and at the same time evoking a sleep spindle. The auditory stimulus S on the other hand is able to evoke a SO, but not a sleep spindle (Figure 6a). A related possibility is that process P evokes a slow oscillation and a processes P_n (or a cascade of processes), which releases a sleep spindle. Auditory stimulus S would also evoke a slow oscillation but not trigger process P_n to trigger a spindle (Figure 6b). Another possibility is that the endogenous process P and auditory stimulus S trigger a different type of SO, where the SO then triggers the sleep spindle directly (Figure 6c) or indirectly via process P_n (Figure 6d) for the endogenous SO but not for the induced SO. K-complexes are isolated SOs that can be evoked through auditory stimuli. While SOs and KCs are considered to be the same phenomenon by some (F. Amzica & Steriade, 1997; Florin Amzica & Steriade, 2002), Cash and colleagues (2009) differentiate between KCs and SOs. They propose that KCs represent a cortical down state surrounded by weaker up-states (S. S. Cash et al., 2010;

Halász, 2016). Considering the two functions proposed for KCs or SOs during N2 sleep (protection against arousal (Jahnke et al., 2012; Laurino et al., 2014) and consolidation of memories (Diekelmann & Born, 2010; Stickgold, 2005; Tononi & Cirelli, 2006)), it seems possible that there are different types of slow oscillations to achieve this function. On the one hand, KCs that only have a strong hyperpolarizing component (neuronal silence) to protect against waking, and on the other hand SOs with a strong depolarizing component (synchronized neuronal firing) for memory consolidation. In the model proposed above, this would correspond to endogenous process P eliciting an SO (Figure 6c and d, top) and auditory stimulus S eliciting a protective KC (Figure 6c and d, bottom) that blocks the release of a sleep spindle. If the closed-loop stimulation paradigm was able to preferentially release 'P-type' SOs by playing into the up-state of an endogenous SO, and open-loop stimulation preferentially released 'S-type' KCs, because the first cue had to be loud enough to reliably trigger an SO/KC, then this could explain why closed-loop contrary to open-loop stimulation had a beneficial effect on memory consolidation. This is only a speculative model of the mechanism coordinating the interplay of SOs and sleep spindles. Further research into this mechanism is needed to fully understand the interplay of nested oscillations.

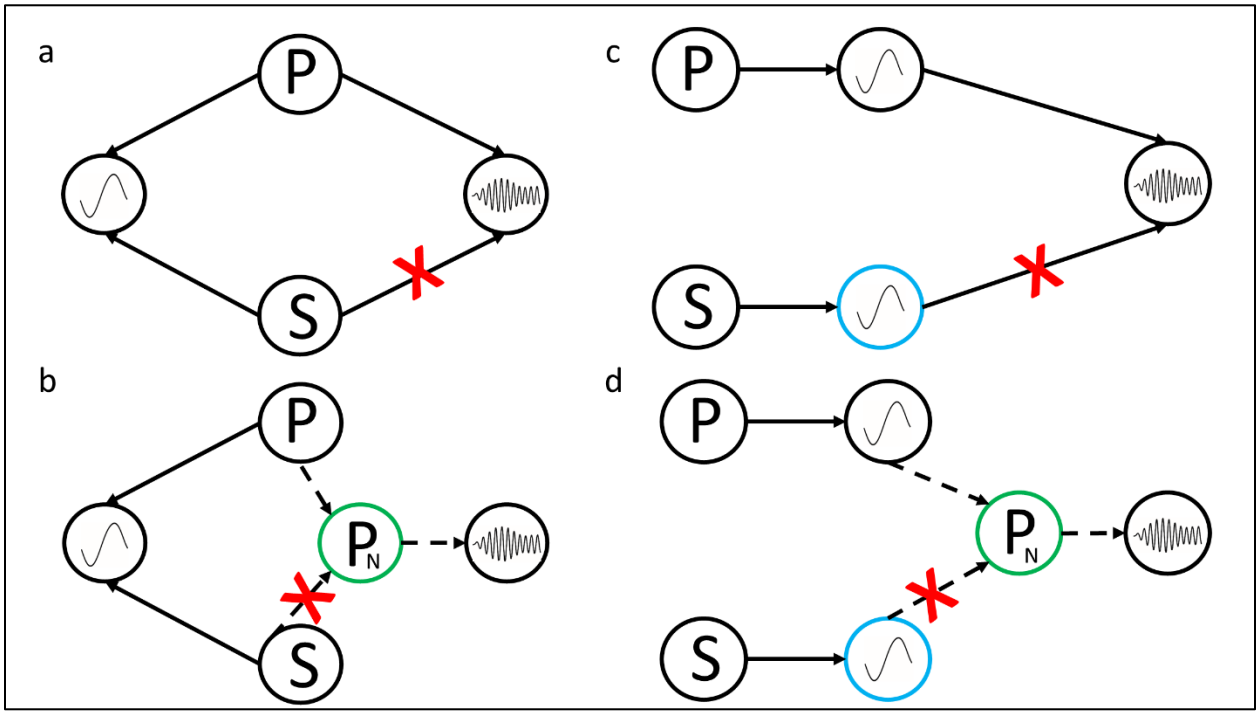

Figure 6| Possible interaction mechanisms between slow oscillations and sleep spindles. a) an endogenous process P evokes an SO. At the same time it evokes a sleep spindle. The auditory stimulus S also evokes an SO but cannot evoke a sleep spindle. B same mechanism as in a) but sleep spindles are not directly evoked from P but through a cascade of processes P_n. Stimulus S is not able to trigger the cascade P_n. c) Process P elicits an SO which in turn evokes a sleep spindle. Stimulus S on the other hand evokes a KC which is not able to evoke a spindle. d) same as c) but SO triggers / KC does not trigger a cascade P_n which releases the sleep spindle.

For ASH, it is not only the functional coupling of SOs and sleep spindles that are integral to memory consolidation. In addition, the nesting of SWRs in the troughs of sleep spindles plays a critical role. It is still an open research question how SWRs are affected by auditory stimulation of SOs. Moreover, it is unclear whether closed- or open-loop stimulation have any effect on the generation of SWRs.

4.3 The Beneficial Effect of Targeted Memory Reactivation

While the studies discussed above used sound cues to optimize the environment within which memory consolidation can take place, i.e. more SOs during NREM sleep, in manuscript III (Göldi et al., 2017) we used TMR to target specific memory traces associated with an auditory cue. Combining the insight from manuscript II (Göldi & Schreiner, 2017) that the endogenous oscillations of the brain are important for preserving their function and the theoretical assumptions of ASH that the SO up-state is the time window for memory consolidation, we implemented a closed-loop SO predicting and stimulus evoking protocol. The goal of this study is to find the optimal time point for targeted memory reactivation. This will in turn lead to a better understanding of the underlying mechanisms governing memory consolidation. A mechanism to improve memory consolidation significantly would also be beneficial to the research of memory consolidation in practice as the size of the memory effect and the number of possible reactivations per session always limit the strength of the results. Additionally, an improvement in TMR could also make the method more applicable in a practical every-day use case for memory improvement. Technically, the closed-loop stimulation detects a potential SO, estimates the onset of the down-to-up-state transition and plays an auditory stimuli (the Dutch word of a previously learned Dutch-German word pair) into the up-slope of a slow oscillation. At this point it is important to distinguish between the closed-loop auditory stimulation (Ngo et al., 2013) discussed above and the closed-loop stimulation presented in manuscript III (Göldi et al., 2017).

While both closed-loop schemes use a similar method to detect SOs, the former plays tones into the peak of the SO up-state to induce a further SO. The latter closed-loop scheme plays a memory cue into the down-to-up-state transition of the SO to induce the reactivation, and thus strengthening, of that memory trace. Manuscript III shows that words cued into the up-state of a SO are better remembered than uncued words. This is in line with the theoretical predictions of ASH. As neurons start to depolarize and fire synchronously, the auditory cue reactivates the related memory trace with a higher chance than random activation. Also SHY would argue that the up-state is the optimal time window for TMR. Synaptic connec-

tions are down regulated during the up-state of the SO. The connections of neurons firing together however are protected from down-scaling. As TMR reactivates a complete memory trace, the trace is protected from down-scaling. The tacit assumption of both hypotheses is that the auditory cue immediately reactivates the corresponding memory trace. Words cued into the down state of a SO did not significantly differ from up-state cued or uncued words. Descriptively the memory performance was between that of up-state cued words and that of uncued words. The assumed mechanism of TMR is that the cue being played into the up-state causes a reactivation of the hippocampal and neo-cortical memory traces and thus strengthens them. This can however not explain why down-state cueing shows a descriptive memory improvement over uncued words. During down-states there is neuronal quiescence and a memory trace should not be easily reactivated. This argument however implicitly assumes that a cue immediately triggers a memory trace reactivation.

Another possibility is that the auditory stimulation first marks the memory trace to be reactivated in the following SO-spindle complex. This would conceptually have a resemblance to marking a stimulus as relevant for the future, which has been shown to improve memory consolidation (Fischer & Born, 2009; Wilhelm et al., 2011). A mechanistic resemblance between these two phenomena is however is not implied here and would be purely speculative. Under this assumption, the probability of tagging a memory trace for reactivation would be highest during a SO up-state and lowest during a SO down-state.

Related to the possibility that a memory trace is marked for reactivation is the question whether TMR only increases the number of reactivations by one, or does reactivation mark the memory trace in general to make it more susceptible to random reactivations. In rats, the cueing of a tone associated with a left or right track increased the chance of a replay event happening in place cells associated with the respective track for up to 10 seconds or until another tone was played (Bendor & Wilson, 2012). This means the tone did not only induce a single replay event. Rather the chance of replay was increased for a longer period. A single reactivation can therefore cause multiple replays of a memory trace. The question then is how often is a memory trace reactivated spontaneously during sleep? If we assume that a memory trace is randomly reactivated much more often than the number of reactivations during TMR (typically ~10 in 'random-state' TMR and ~5 in the study presented in manuscript III), then the effect of a single additional reactivation of a memory trace would be negligible. If spontaneous reactivations of a memory trace happen on the order of hundreds or even more, then the addition of 10 more reactivations through TMR could not lead to a memory improvement. However, if one successful reactivation tags the memory trace for repeated reactivations, 10 reactivations could lead to hundreds of additional replays. This would make

a positive memory effect viable. If however spontaneous reactivation of a specific memory trace during SOs is very sparse e.g. tens of reactivations, then a single additional successful reactivation could have a measurable impact on memory performance. It would therefore not be necessary for a reactivation to tag a memory trace for multiple reactivations. A single and immediate replay would be enough. It is unclear whether TMR enhances memory consolidation because it increases the chances of a memory cue being replayed multiple times, or if it only promotes a single additional replay. This is still an open research question. Addressing it could lead to a better understanding of how TMR works and how it could potentially be enhanced.

4.4 Post-Stimulus Sleep Spindles and Theta Activity as a Marker of Successful Reactivation

For the successful up-state cued words, we find an increase in spindle power after 1 second. This corresponds to the upstate of the oscillation following the SO into which the stimulus was cued. As discussed in manuscript II (Göldi & Schreiner, 2017) spindle activity was a necessary neural marker for memory improvement. Down-state cueing on the other hand showed no difference in spindle band activity between remembered and non-remembered words. It is important to note that the spindle band activity 1 second after cue onset did not differ between up- and down-state cues in general. It is therefore not the case that sleep spindles could not be generated 1 second after the auditory down-state cue. Rather there was the same amount of spindles for both cue types. For up-state cues, these are just more specific to successful reactivations. This would indicate there are other important mechanisms at play and spindle activity is not a sufficient marker for successful memory reactivation. After cue onset, the negative post-stimulus peak highly resembles an endogenous ~1 Hz slow wave. The down-state cues disrupt the natural continuation of the spontaneous slow wave. As a result, the evoked response of up- and down-state cues is near-perfectly aligned. If this is indeed the critical window (as argued above), where the memory trace is actually reactivated this similarity in temporal evolution could explain the slight improvement of memory cues during down-state cueing over uncued words.

In the time window of approximately 1 to 1.5 seconds, we also find an enhancement of theta activity for remembered up-state cues versus non-remembered cues (Manuscript III). The down-state again shows no difference between remembered and non-remembered words. Theta activity was also found in (Groch et al., 2017; Lehmann et al., 2016; Schreiner, Lehmann, et al., 2015; Schreiner & Rasch, 2015a) to be a marker for successful memory reactivation. Manuscript III confirms the relevance of theta for sleep. Man-

uscript I (Schreiner, Göldi, et al., 2015) finds theta activity to be a marker for successful memory recognition during wake (see also discussion in section 'The Critical Role of Theta Activity for Memory Consolidation'). Also theta power is a marker for encoding memories (Axmacher et al., 2006; Huerta & Lisman, 1995; Hyman et al., 2003) and successful memory retrieval (W. Klimesch et al., 2001; Nyhus & Curran, 2010) during wake. It could therefore be argued that theta power is a marker for a successful access to a memory content in both wake and sleep. The exact mechanistic purpose of theta activity during sleep however remains open to research.

4.5 The Relevance of REM Sleep for Memory Consolidation

Theta activity in sleep research has more typically been associated with REM sleep (Buzsáki, 2002) and is generated within CA1 area of the hippocampus (Goutagny et al., 2009). Although there is strong evidence for a role in hippocampal theta activity during wake (Benchenane et al., 2010; Colgin, 2011; Fujisawa & Buzsáki, 2011) especially in conjunction with gamma oscillations (Colgin, 2015; Lega, Burke, Jacobs, & Kahana, 2016; Lisman & Jensen, 2013), the evidence for an involvement of REM sleep theta remains scarce (Rasch & Born, 2013). During REM sleep there is a reduced coordinated information flow between hippocampal input and output regions and hippocampal and neocortical regions (Rasch & Born, 2013). These high levels of local information processing are thought to favor synaptic consolidation (Diekelmann & Born, 2010) and have been associated with synaptic down-scaling ((Grosmark, Mizuseki, Pastalkova, Diba, & Buzsáki, 2012); as proposed by ASH, SHY leaves the memory function of REM sleep open at this point (Tononi & Cirelli, 2014)). In manuscript III we did find a positive correlation of time spent in REM sleep with memory performance. However, this was only true for the down-state cued words. The ASH proposes that NREM sleep promotes system consolidation by allowing for a dialog between hippocampal and neocortical brain regions. However, only if the process is not interrupted by a stimulus in the critical 1.5 second time window after the cue is presented (Schreiner, Lehmann, et al., 2015). If we assume that during the down-state cueing the memory trace is reactivated, but it cannot be stabilized within the critical time window (Schreiner & Rasch, 2017), the hippocampal memory trace could still be consolidated within the hippocampus (perhaps by way of down-scaling) during REM sleep. This could explain why, although the typical oscillatory markers (theta and sleep spindles) for successful memory reactivation are not found (perhaps due to the environment of the endogenous brain state), there is still successful hippocampal consolidation. In that case, NREM could be seen as the time for inter-brain region communication and REM sleep as a time for intra-region communication. During recall participants would therefore not benefit from a strengthened cortical memory representation but rather from a strengthened hippocampal

memory trace. This proposed mechanism does depend on down-state cued words being marked within the hippocampus during cueing, albeit there being no oscillatory sign for it. In manuscript III we also find a marginal negative correlation of time spent in REM sleep and memory performance with uncued words. Following the argumentation just presented, this could be explained by the fact that during REM sleep the memory traces of uncued words are less protected from hippocampal down-scaling than cued words. The memory traces of uncued words are therefore reactivated less during NREM sleep system consolidation is minimal. During subsequent REM sleep the hippocampal memory traces is down-scaled. Therefore, there is no enhancing and a subsequent down-scaling effect. Down-state cued words also experience limited system consolidation during REM. Due to the cue the memory trace is however 'marked' in the hippocampus (perhaps by reactivation without subsequent transfer to the neo-cortex) and therefore protected from downscaling (as proposed by SHY) during REM. There is no enhancement but a protection from down-scaling, leading to a slight improvement over uncued words. Up-state cued words benefit mostly from system consolidation and are additionally protected during subsequent down-scaling. Memory performance shows no correlation with time in REM and is higher than for uncued words and slightly better than for down-state cued words. It is important to note that the model proposed here is speculative and needs thorough scientific testing. Especially an answer to the question of where (i.e. hippocampal or cortical) the memory traces are located, that are accessed during retrieval, would provide further insights.

4.6 The Efficacy of Closed-Loop Targeted Memory Reactivation

The question of whether the additional complexity of using a closed-loop TMR paradigm is justified by an increased memory performance must be asked. Overall we find that up-state TMR presented in manuscript III does not exceed 'random-state' TMR benefit (~10 percent) found in earlier studies (Schreiner, Lehmann, et al., 2015; Schreiner & Rasch, 2015a). The earlier studies probably made up for the lack of optimal cues by number of cues, which was double (~10) compared to the current study. In addition, as this study has shown, even non-optimal cueing (i.e. not up-state cueing) has a slight positive effect on memory performance compared to no cueing. In practice, this would mean that while the up-state may be the optimal time to cue an auditory stimulus, the technical effort and the reduction in repetitions may actually outweigh the performance gain of the method. Especially for a practical every-day application of TMR, the setup should be as simple and unobtrusive as possible.

Manuscript IV presents a first attempt of a simple platform to aid vocabulary learning with TMR during sleep over multiple days. Surprisingly, there was no benefit of cueing across the whole four-day experiment. A more detailed analysis showed that there was no cueing effect during the first two nights. Only the third night showed a beneficial memory effect for cued words relative to uncued words. A possible explanation could be the participants' sleep quality. This was reported to be worse for the first two experimental nights and returned to pre-experimental levels for the third night. Looking further, participants that reported waking during the first night because they were disturbed by the auditory stimulus material showed no positive memory effect of cueing on any of the nights. Indeed, they even showed a trend for selective worsening of cued words during the first night. This result was also found in a previous study conducted at home during a single night (not published) and during the piloting phase (21 participants) of the current study. This would indicate that there is not only a restrictive effect of TMR in an uncontrolled environment on sleep and consolidation in general, but also an actual selective negative effect for cued words when sleep is disrupted. Contrary to controlled lab experiments, there can be many confounding factors at play here. These will be discussed next.

First, the participants had a low learning level (~38%) on the first day. In previous studies (Göldi et al., 2017; Schreiner, Lehmann, et al., 2015; Schreiner & Rasch, 2015a), the participants had a learning level of about 50% prior to sleeping. Recent studies have shown that the benefit of TMR during sleep is stronger for higher learning level (Creery, Oudiette, Antony, & Paller, 2015) or prior knowledge (Groch et al., 2017). To test whether this had an effect on the cueing success we matched participants for nights 1 and 3 based on their learning level (~50%). There was still no effect of cueing during night 1 and a positive cueing effect for night 3 remained. If differing learning level had been the explanation for unsuccessful TMR during the first nights of the experiment, then the matched groups should have both had a cueing effect. As this is not the case, learning level was not the driving reason for the ineffectiveness of TMR in this experiment.

Second, contrary to earlier experiments conducted in the lab using the same experimental paradigm (Göldi et al., 2017; Schreiner, Lehmann, et al., 2015; Schreiner & Rasch, 2015a), there was no control over when (in which sleep stage) the auditory cues were presented. It may have been that the unfamiliarity of sleeping with headphones and hearing auditory stimuli during the night has led to a temporal shift in sleep stages. Under lab conditions the sleep EEG is constantly monitored and TMR can be limited to specific sleep stages. In this experimental setup there is no way to verify in which sleep stage the stimuli were actually cued. This would require constant automated monitoring of the sleep EEG at home with a coupled

auditory cue release (similar to the setup described in manuscript III). This is however, beyond a simple and easy to use TMR platform for everyday use and needs to be investigated in future research.

The unavailability of constant sleep EEG monitoring is also a confounding factor for arousal detection. In the lab environment, cueing can be paused as soon as an arousal is detected. This is not possible in the simple setup presented in manuscript IV. However, given that subjects who reported waking during the first night due to auditory cues showed detrimental effects for cued words, this may be a crucial point. It is possible that the memory trace that is reactivated during sleep becomes labile and susceptible to manipulation (McGaugh, 2000; Nader & Hardt, 2009; Tronson & Taylor, 2007). If waking due to the auditory cue prevents (re)consolidated immediately (Schreiner & Rasch, 2017) or during subsequent REM sleep (Rasch & Born, 2013) and as discussed above, this could lead to negative effects for the memory trace in question. Again, future research needs to address this point to gain a better understanding of the state of a memory trace after it has been reactivated during sleep.

Finally, the uncontrolled nature of this experiment with respect to objective sleep parameters makes it difficult to pinpoint the exact cause (or multiple causes) for why the TMR failed during the first two nights. It has also led to interesting questions that remain to be addressed in future research and that will lead to a better understanding of the memory consolidation process in general. The success of TMR during the third night of the experiment points to the need for a reactivation study over longer periods. It remains to be experimentally shown that on consecutive nights, the positive memory effect of cueing remains stable and at which point no further memory improvements can be achieved through TMR. The positive effect of TMR after the third night does encourage the hope that a simple TMR system suitable for daily use is achievable in the near future.

4.7 Recall of Hippocampal or Cortical Memories?

The TMR studies presented in this thesis all ascribe the beneficial effect on memory consolidation to the mechanisms proposed by ASH. However, then the question arises what kind of memories are actually being accessed during wake recall (the same question would arise if all studies argued in favor of SHY): Hippocampal memories or neo-cortical memories? Related to this, is the question if we are seeing the effects of synaptic consolidation or system consolidation during successful memory retrieval in wake. Indeed, as discussed above, it may be different for the individual memory trace and may depend on different forms of reactivation: Some words remembered might be more hippocampus reliant and others more cortex reliant. Synaptic consolidation would have changed the strength of already existing memory traces (presumably in the hippocampus). Synaptic consolidation changes the structure of synapses on a local

level. It works within minutes to hours. System consolidation would have integrated the memory into preexisting knowledge networks. System consolidation is the transfer of information from hippocampal to a more long-term neo-cortical memory system. It works on the time scale of days to weeks, up to a lifetime. Considering that, except for the study presented in manuscript IV, all studies presented in this thesis, indeed, to the knowledge of the author, all studies on memory reactivation published to date only look at a single night (or an afternoon nap) of reactivation. Considering the time scale, it therefore seems unlikely that full system consolidation is achieved and the memory trace is hippocampus independent. However, this is not necessary for memory improvement. As the transition to hippocampus independent memory is gradual, already a slight increase of cortical strength in the memory trace can lead to a higher chance of activating the cortical and hippocampal memory trace, leading to a better performance in memory recall. As stated above, further research into this area could lead to a better understanding of memory consolidation processes.

4.8 ASH versus SHY

As indicated earlier in this thesis, the two eminent hypotheses regarding the function of sleep will be contrasted here. While the manuscripts presented have argued the effects of consolidation from the perspective of ASH, the argumentation in no way speaks against SHY. ASH argues that TMR works by actively reactivating memory traces and therefore strengthens the synaptic pathways making up that memory trace relative to other synaptic connections. SHY on the other hand argues that all synaptic connections are downscaled during sleep. Through TMR, memory traces are marked for protection from downscaling. In combination with the global downscaling, this also leads to stronger connections of reactivated memory traces relative to other synaptic connections (Nere et al., 2013). On a behavioral level, it is not possible to judge which mechanism is at work during TMR. Both hypotheses can explain the beneficial effect of TMR during sleep, even though they propose nearly opposing mechanistic processes. On the oscillatory level, they are also difficult to separate. While the nesting of SOs, spindles and ripple events and their relation to memory consolidation has been shown experimentally and in accordance with ASH (Staresina et al., 2015), it is absolutely viable that at the same time down-scaling mechanisms are at work in the cortex and perhaps even in the hippocampus (as is proposed by SHY). As discussed above however, it is possible that hippocampal synaptic consolidation occurs during REM sleep (proposed by ASH, left open by SHY). Whether down-scaling during REM sleep is restricted to the hippocampus or indeed if down-scaling in the hippocampus is restricted to REM sleep remains an open research question.

To decide which hypothesis better describes the mechanisms underlying memory consolidation is very difficult to judge, because they operate at different levels. It may therefore well be that further insights into the mechanisms underlying memory consolidation will allow the two hypotheses to be united in significant parts. SHY does not propose a specific mechanism by which memory consolidation must happen, but rather proposes that the overall purpose of sleep is to reinstate synaptic homeostasis (Tononi & Cirelli, 2012). ASH on the other hand proposes a very specific mechanism by which memory consolidation works (Rasch & Born, 2013). Both hypotheses are backed by a wealth of scientific evidence. It therefore seems likely that both hypotheses describe a part of the complex processes going on in the brain during memory consolidation. The mechanisms may be at work at the same time or alternate in a complex interplay that is yet to be fully understood.

4.9 Conclusion

This thesis has presented and discussed four manuscripts related to the study of the oscillatory mechanisms underlying memory consolidation. The critical role of theta activity, both in wake as a persistent oscillatory marker for memory encoding and in sleep as a marker for successful memory consolidation has been shown. Additionally, the importance of the interplay of SOs, sleep spindles and theta activity has been reinforced. Especially the SO up-state could be confirmed as being the optimal time window for TMR. A tentative link between memory consolidation and REM sleep has been established and discussed. On a behavioral level, the efficacy of TMR to boost vocabulary learning under supervised lab conditions has been confirmed. However, unsupervised TMR over multiple days at home did not yield the expected positive TMR effect. While evidence for some answers have been provided many questions remain and new ones arise. The question of how exactly theta as a memory specific oscillation interacts with typical sleep specific oscillations remains to be answered. Indeed, a better understanding of the interactions of different oscillations in general could lead to insights about brain processes. Additionally, many questions about the interplay of NREM and REM sleep remain open. Further insights here could in general lead to a better understanding of how the brain uses distinct state to complete different tasks necessary for its proper functioning.

Sleep and memory research remains full of questions and will remain to do so for a long time as the complex brain, piece by piece reveals its secrets to us. And whether it is ASH, SHY or another hypothesis that will ultimately prevail in the question about sleeps role, it will surely integrate significant portions of the other.

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6 Curriculum Vitae

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2011 – 2014	Master of Science Informatics: Multimodal and Cognitive Systems
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2009 – 2011	Bachelor of Science Informatics: Neuroinformatics
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2002 – 2008	Studies in Electrical Engineering and Computer Sciences
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7 List of Publications

Journals

M. Göldi, E. van Poppel, B. Rasch & T. Schreiner (2017). “Cueing memory during sleep is optimal during slow-oscillatory up-states.” bioRxiv, 1–24. https://doi.org/10.1101/185264

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M. Göldi, S. Ackermann, A. Papassotiropoulos , D. J.-F. de Quervain , B. Rasch (2015). "Large Scale Sleep EEG Data Analysis of Slow-Waves and its Cross-Frequency Coupling with Memory-Related Oscillations", Psychologie und Gehirn 2015 (PuG2015), June 4-6, Goethe University Frankfurt, Germany.

M. Göldi, S. Ackermann, A. Papassotiropoulos , D. J.-F. de Quervain , B. Rasch (2015). "Large Scale Sleep EEG Data Analysis of Slow-Waves and its relation to Episodic Memory Consolidation", Swiss Society for Neuroscience Annual Meeting 2015 (SSN2015), January 24, University of Fribourg, Switzerland, p.50.

M. Göldi, S. Ackermann, A. Papassotiropoulos , D. J.-F. de Quervain , B. Rasch (2015). "Large Scale Sleep EEG Data Analysis of Slow-Waves and its relation to Episodic Memory Consolidation", CRPP Sleep and Health Symposium, January 15-17, University Hospital Zurich, Switzerland, p.27.

S. Miyashita, M. Göldi, K. Nakajima (2011). "Role of morphology on two dimensional magnetic self-assembly.", The 2nd International Conference on Morphological Computation (ICMC2011), September 12-14, 2011, Venice p.58-60.

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A. Tientcheu, M. Göldi, S. Miyashita, R. Fuchslin, and R. Pfeifer (2010) "Achieving Self-Sorting in Self-Assembly Systems", 4th International Conference on Cognitive Systems (CogSys2010), January 27-28, 2010, ETH Zurich, Switzerland, p. 93.

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Other

M. Göldi " Ist Schlaf noch zeitgemäss", Thema im Fokus, Nr 130, December 2016, Zürich, Switzerland

M. Göldi " Roboter im Gesundheitswesen: Was Roboter Können – was sie nicht können", Thema im Fokus, Nr 115, June 2014, Zürich, Switzerland